Advances and Emerging Opportunities in Type 1 Diabetes Research: A Strategic Plan

Developed Under the Auspices of the Diabetes Mellitus Interagency Coordinating Committee

DRAFT DOCUMENT

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INTRODUCTION

Overview, Burden, and Impact of Disease

This Strategic Plan focuses on type 1 diabetes—the form of the disease in which the body's immune system mistakenly destroys the cells that produce insulin, a hormone that regulates the amount of glucose (sugar) in the blood and is essential for life. In the other major form of diabetes—type 2 diabetes—the cells of the body lose their ability to respond to insulin even though patients produce the hormone. Although the underlying causes of type 1 and type 2 diabetes differ, both forms of the disease share the same possible complications, which include blindness, kidney failure, nerve damage, lower limb amputations, heart disease, and stroke. Type 1 diabetes can be more serious and costly for patients because it tends to strike earlier in life. For example, while type 2 diabetes increases the risk of heart disease 2-4 fold (1), heart disease risk is increased up to 10-fold in type 1 diabetes compared to the general age matched population (2, 3). Importantly, the longer a person has diabetic complications, the more severe, difficult-to-treat, and costly they can become. Thus, an early diagnosis of type 1 diabetes can set the stage for a lifetime of living with and medically managing the disease complications. With respect to quality-of-life, dreaded complications can diminish the vitality of childhood and adolescence, as well as the prime productivity of young adulthood. Patients and their parents often wait anxiously to receive test results of their eye and kidney function. A broken blood vessel in the retina, or the finding of protein in the urine, can be the first sign that a relentless complication of the disease has emerged, and that grueling and costly treatments are in the near future. Even with recent advancements in treatment, type 1 diabetes is estimated to lower average life expectancy by 15 years (4). For childhood-onset cases, greater than 15 percent of patients with type 1 diabetes will die by age 40 (4). Thus, early onset type 1 diabetes has major adverse impacts on patients and on society because of its extremely high personal and economic costs. Because both type 1 and type 2 diabetes involve derangements in the body's system for maintaining appropriate blood sugar levels, and because both also share the same complications, it is likely that research directed toward one form of the disease would have beneficial application to the other form as well.

Diabetes can affect nearly every organ system in the body. Collectively, both type 1 and type 2 diabetes are thus an enormous public health challenge in the United States. The following indicators of the burden of diabetes apply to both forms of the disease.

- Patients with diabetes have an increased risk of heart disease and heart attacks, stroke, high blood pressure, kidney failure, blindness, nerve pain and other neurological problems, limb amputation, chronic wounds and skin ulcers, gum disease, and pregnancy-related problems.
- In the past 25 years, the number of people with diabetes has more than doubled to 20.8 million (5, 6), or 7 percent of the total U.S. population (5).
- Evidence now suggests that 1 in 3 Americans born in 2000 will develop diabetes during his or her lifetime (6).
- Diabetes is the sixth leading cause of death in the United States, resulting in more than 73,000 deaths in 2002 (7). More than 224,000 individuals die annually from diabetes-related complications, a number that is considered to be significantly underreported (5).

- The problem of diabetes extends globally. The World Health Organization estimates that 1,125,000 people worldwide will die from diabetes in 2005 (8). Overall, the risk of death for individuals with diabetes is approximately double that of people without diabetes (5).

The burden of both forms of diabetes extends far beyond mortality. In the United States each year, 12,000-24,000 individuals become blind as a result of diabetic eye disease (5) and approximately 82,000 people undergo diabetes-related amputations (5). Encouragingly, declines in the incidence of end-stage renal disease due to diabetes are being noted for the U.S. population, in reports from the United States Renal Data System. These gains are most noteworthy in diabetic patients under age 30 (most of whom have type 1 diabetes) and are restricted to Caucasians and not observed in African-Americans (9). Despite this encouraging data, in 2003, 45,330 Americans with diabetes began treatment for irreversible kidney failure (end-stage renal disease), while 165,113 individuals with failed kidneys needed chronic dialysis or a kidney transplant to remain alive (9). In this country, adults with diabetes have rates of heart disease that are 2-4 times higher than adults without the disease (1). Complications extend into high blood pressure, dental diseases, and neurological and pregnancy-related problems. These complications are far reaching.

The financial burden of diabetes is tremendous. The direct and indirect costs associated with both forms of diabetes in the United States in the year 2002 were estimated to be $132 billion (5). The average annual health care costs for a person with diabetes are $13,243, which is 2.4 times greater than those for an individual without diabetes (10). In 2002, 11 percent of national healthcare expenditures were directed to diabetes care (10). The costs of treating the complications of diabetes, which both forms of the disease share in common, account for much of the health care costs associated with the disease. Although estimates of the rates of diabetes have increased since 2002, the associated cost estimates have not yet been revised; hence, the economic data given here are conservative. Clearly, the economic and societal burden of diabetes has a profound impact on American society.

Incidence and Prevalence of Type 1 Diabetes

The incidence (the number of new cases) and prevalence (the total number of cases) of type 1 diabetes are not precisely known. In the U.S., it is estimated that approximately one in every 400-600 children and adolescents has the disease (5), with more than 13,000 young people diagnosed each year (11). In total, about 30,000 people are diagnosed with type 1 diabetes annually (11). Once diagnosed, individuals must manage the disease for the remainder of their lives. There is no cure.

Key Features of Type 1 Diabetes

Type 1 diabetes is an autoimmune disease, in which the body's own immune system mistakenly attacks and destroys specialized cells of the pancreas called beta cells. Beta cells are found within tiny clusters called islets, and are the body's sole producer of the hormone insulin. Insulin is required for survival; it sends signals to the body's cells and tissues telling them to absorb glucose to use as a fuel. Without this vital hormone, the cells and tissues do not absorb glucose and patients can starve to death, despite having high levels of glucose in their bloodstream. An

interplay of genetic and environmental factors is responsible for the onset of type 1 diabetes (as well as type 2 diabetes). Having a family member with the disease puts one at higher risk.

Type 1 diabetes differs from type 2 diabetes--which is more commonly diagnosed in adulthood, is strongly associated with overweight and obesity, and disproportionately affects minority populations. Although type 1 diabetes patients require externally administered insulin to survive, type 2 diabetes patients may be treated with medications that make their tissues more sensitive to insulin or enhance insulin production or, in some cases, with insulin itself. Despite the important differences between type 1 and type 2 diabetes, research on type 1 diabetes has contributed greatly to improved management of both forms of the disease. For example, a landmark clinical trial in type 1 diabetes, the Diabetes Control and Complications Trial (DCCT), proved that intensive glucose control can prevent or delay damage to the small blood vessels in the eyes, kidneys, and nerves (microvascular complications). The findings of this trial paved the way to studies that replicated these impressive results in type 2 diabetes patients. Most recently, the findings from this trial were extended to large blood vessel damage that can lead to heart attacks and strokes (macrovascular complications). Thus, because of pioneering research in type 1 diabetes, close control of blood glucose levels is now a keystone to the medical management of both forms of the disease. Moreover, the initial trial in type 1 diabetes also established HbA1c--a method for measuring blood sugar levels over time--as a basis for approval of new diabetes drugs by the Food and Drug Administration (FDA). This test subsequently became an important outcome measure for future clinical trials of both type 1 and type 2 diabetes. The use of HbA1c as an outcome measure was the basis for approval by the FDA of improved forms of insulin, as well as many other new drugs for type 2 diabetes.

Treatment Options and Challenges

The treatment of type 1 diabetes patients was revolutionized in 1921 with the discovery of insulin by a group of researchers at the University of Toronto. To this day, insulin therapy continues to save the lives of type 1 diabetes patients by replacing the essential hormone that their body no longer adequately produces. However, insulin therapy, whether through injections or via a pump, is not a cure. Patients must carefully monitor their food intake and physical activity in order to manage the disease. They must perform painful “finger sticks” multiple times per day to draw blood and test their glucose levels. Based on this monitoring, they often give themselves several shots of insulin per day, or calculate the correct amount of insulin to administer through their insulin delivery pumps. This regimen is not just “once in a while;” it is every day of their lives. As many patients and their parents say: “There is never a day off from diabetes.” Moreover, no matter how vigilant patients are at regulating their blood glucose levels, they can never achieve the fine-tuned regulation provided by a healthy pancreas, which exquisitely senses and responds to insulin needs with precise timing.

Because of the inadequacies of insulin treatment, type 1 diabetes patients are susceptible to harmful fluctuations in their blood glucose levels—abnormally high blood glucose (hyperglycemia) or dangerously low blood glucose (hypoglycemia). Both of these conditions can be life-threatening in extreme cases. In the case of a very young type 1 diabetes patient who cannot self-monitor, parents must assume the role that is no longer performed by the pancreas. Parents often forego restful sleep because they are “on watch” to ensure that their child’s blood glucose levels do not fall dangerously low in the middle of the night.

Approaches for Preventing or Reversing the Disease

Currently, there are no known methods to prevent type 1 diabetes, but recent clinical trials suggest that it may be possible to reverse or slow the rate of loss of the insulin producing beta cells in patients newly diagnosed. While the environmental factors that may play a role in triggering type 1 diabetes remain to be defined, key genes that increase the risk of type 1 diabetes have been identified. Genetic tests in combination with blood tests to detect antibodies directed against the insulin producing beta cells can predict development of type 1 diabetes, allowing new strategies for prevention to be tested. Key strategies for preventing much of the burden of the disease include early detection, improved methods and delivery of care, and new interventions. With the number of individuals with diabetes increasing, the associated societal and economic burdens will continue to rise. Yet, there are many positive developments, including reports showing that life expectancy for patients with type 1 diabetes is increasing (12). A key finding of NIH-supported research is that intensive control of blood glucose levels can dramatically prevent or delay the development of disease complications. Now, it is essential to find more effective ways to achieve blood glucose control. Progress is also being made in the area of cell-based research that could lead to ways to replace or restore a patient's insulin-producing capacity. With continued, vigorous research, new strategies may be developed to prevent type 1 diabetes in those at-risk, restore insulin independence in patients already diagnosed, and prevent the development of disease complications. It is through research in these and other avenues that the burden of type 1 diabetes on people and the Nation can be lifted.

Goals of Type 1 Diabetes Research

The promise of a cure for type 1 diabetes can only be realized through the vigorous support of scientific research. Type 1 diabetes research supported by the NIH is focused around six overarching research goals.

Goal I: Identify the Genetic and Environmental Causes of Type 1 Diabetes
Goal II: Prevent or Reverse Type 1 Diabetes
Goal III: Develop Cell Replacement Therapy
Goal IV: Prevent or Reduce Hypoglycemia in Type 1 Diabetes
Goal V: Prevent or Reduce the Complications of Type 1 Diabetes
Goal VI: Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes

Goal I: Identify the Genetic and Environmental Causes of Type 1 Diabetes

Type 1 diabetes has a strong genetic basis that is modified by environmental factors. Type 1 diabetes is a "polygenic" disease, which means that it arises from the interaction of variations in multiple genes. Research has already identified some genes that are important in the development of type 1 diabetes. However, researchers have not yet found all of the genes that can play a role in disease development. Identification of key genes will not only help to predict who will develop the disease, but also aid in the development of new prevention strategies.

In addition to genes, the environment has also been found to play an important role in type 1 diabetes disease development. Potential environmental triggers are thought to include viruses, dietary factors, environmental toxins, and psychological stress. To date, no single trigger has been conclusively identified. Research to identify the key environmental trigger(s) could be used to prevent the disease in genetically susceptible persons.

Goal II: Prevent or Reverse Type 1 Diabetes

A way to attack type 1 diabetes is to stop it before it ever starts. Preventing the disease means that patients would not require insulin administration or develop life-threatening disease complications. While recent clinical trials have suggested that further loss of insulin production can be slowed in patients with newly diagnosed type 1 diabetes, research has not yet identified an effective disease prevention strategy. However, the ability to identify at-risk individuals permits promising strategies for prevention to be tested in rigorously designed clinical trials. Further research and increasing knowledge about what goes wrong with the immune system will facilitate the discovery of novel ways to prevent autoimmunity, and thus prevent disease onset.

In addition to preventing the disease before beta cell destruction starts, it is also important to conduct research to prevent further beta cell destruction in newly-diagnosed patients. Research has shown that, after patients are diagnosed with the disease, they still have some beta cell function and can produce C-peptide, a useful measure of endogenous insulin production. Furthermore, clinical studies have demonstrated major benefits of residual beta cell function in type 1 diabetes patients even though the patients require insulin therapy. For example, the NIDDK-supported Diabetes Control and Complications Trial (DCCT) demonstrated that higher and sustained levels of C-peptide were associated with reduced incidence of long-term disease complications of the kidneys and the eyes and with reduced hypoglycemia. This type of evidence suggests that preserving patients' remaining beta cell function could have dramatic, long-term health benefits. Already one agent has been shown to preserve beta cell function in new onset type 1 diabetes. To prevent or reverse beta cell destruction in newly-diagnosed patients, further research efforts are required to identify and test additional strategies that may provide more durable benefits and few side effects.

Goal III: Develop Cell Replacement Therapy

Type 1 diabetes patients require insulin therapy because their immune systems have destroyed their pancreatic beta cells. A real "cure" for this disease could be achieved by replacing those missing cells, and scientists are aggressively pursuing this avenue of research. A major breakthrough occurred in 2000 when researchers at the University of Alberta in Edmonton, Canada, reported that type 1 diabetes patients achieved insulin independence after transplantation with islets from two-to-four donor pancreata and treatment with a novel immunosuppressive regimen that omitted the widely used glucocorticoid drugs that are toxic to islets. A major barrier limiting the widespread use of islet transplantation is the shortage of islets available for transplantation. The diabetes research community believes that there is significant potential in the use of human embryonic[1] and tissue-specific adult multipotent progenitor cells in deriving a host of differentiated cell types, including insulin-producing beta cells. Understanding the underlying molecular mechanisms of beta cell biology, and how mature beta cells are formed from stem/progenitor cells, could help to overcome this barrier. Furthermore, as noted

[1] The NIH supports human embryonic stem cell research consistent with federal funding policies.

previously, recent research has shown that type 1 diabetes patients have some remaining functional beta cells. Therefore, research on the mechanisms controlling islet cell growth and regeneration could lead to novel therapies designed to stimulate beta cell growth *in vivo*. Another major barrier that prevents islet transplantation from being a widespread treatment option for type 1 diabetes patients is the need for lifelong immunosuppressive drug treatments that are currently required to prevent rejection of transplanted islets. Research to identify ways to overcome the need for immunosuppressive treatment, or to identify less-toxic immunosuppressives, can help to make islet transplantation a reality for greater numbers of type 1 diabetes patients.

Goal IV: Prevent or Reduce Hypoglycemia

Hypoglycemia, or low blood sugar, is perhaps the most distressing acute complication of type 1 diabetes. Hypoglycemia can occur with missed meals, exercise, or when there is too much insulin in the body, which causes glucose to fall to dangerously low levels. Too little glucose means that the body, and particularly the brain, cannot function at its normal capacity. The immediate effects of hypoglycemia can include changes in cardiovascular and nervous system function, cognitive impairment, increased risk for unintentional injury, coma, and sometimes even death. In some cases, patients are not aware that their blood sugar is dangerously low. This syndrome is called "hypoglycemia unawareness." It is characterized by the loss of the warning symptoms that alert patients that it is time to eat before their blood sugar level falls too low.

A severe limitation to the practice of intensive glucose control to prevent disease complications is the potential for acute episodes of hypoglycemia. It is estimated that patients on intensive treatment have two hypoglycemic episodes per week, *versus* one episode if they are treated less intensively (13). Because intensive glucose control dramatically reduces the risk of long-term disease complications, it is imperative to pursue research to overcome this major obstacle to achieving tight glucose control. Research to improve glucose monitoring and insulin delivery, with the aim of reducing hypoglycemic episodes, can also have a major impact on patients' quality-of-life until cell replacement therapy becomes a viable option for type 1 diabetes patients.

Goal V: Prevent or Reduce the Complications of Type 1 Diabetes

Nearly every organ in the body is adversely affected by type 1 diabetes. Throughout the course of a patient's life, the persistent elevation in blood glucose levels, despite insulin therapy, damages vital organs including the heart and kidneys. The longer that a person has the disease, the more likely it is that he or she will develop these severe complications. Because type 1 diabetes patients are often diagnosed in childhood and adolescence, they may develop complications at a young age.

The good news regarding preventing or delaying the onset of complications has come in the form of a landmark NIH-supported research study, the Diabetes Control and Complications Trial (DCCT). Completed in 1993, the trial compared the relationship between intensive *versus* conventional treatment of blood glucose levels and the development of disease complications. The DCCT proved conclusively that intensive therapy reduces the risk of microvascular (small blood vessels) complications, such as diabetic eye, kidney, and nerve disease. Nearly all patients who participated in the DCCT volunteered to continue to be followed in the Epidemiology of Diabetes Interventions and Complications (EDIC) study, which began in 1994. The

EDIC/DCCT researchers continue to report remarkable long-term benefits of intensive blood glucose control in preventing or delaying complications of the eyes, kidneys, and the heart. However, given the limitations and difficulties of current therapies and technologies for achieving good glucose control, even most participants in the EDIC study are not able to achieve the levels of control associated with reduced complications. Thus, other approaches are needed to prevent progress of complications, and research is still needed to provide insights into the underlying molecular mechanisms of diabetes complications in order to develop new therapeutic approaches.

Goal VI: Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes

Research on type 1 diabetes spans a broad range of scientific disciplines, including endocrinology and metabolism; immunology; genetics; population-based studies; neuroscience; cell, developmental and vascular biology; and the physiology of the heart, eyes, kidneys, and urologic tract, and the central and peripheral nervous systems. Propelling research progress on the understanding, prevention, and cure of type 1 diabetes requires a cadre of scientists with diverse research training and expertise. Furthermore, it is critical for basic scientists and clinical researchers to work together to translate research findings from the bench to the bedside, and from the bedside to clinical practice, in order to achieve real improvements in patients' health and quality-of-life.

In addition, new technologies that have emerged over the past few years make this an exciting time to be involved in scientific research. It is important to apply these new and emerging technologies to type 1 diabetes research. For example, "proteomics" involves the use of novel, integrated technologies to identify and quantitate proteins and study their interactions. Identifying how protein expression changes over the course of type 1 diabetes onset and progression can help researchers understand the underlying disease processes, develop biomarkers of disease onset and progression, and propose and test novel prevention and treatment strategies. Type 1 diabetes research stands to benefit greatly from the application of proteomics and many other new technologies.

NIH Type 1 Diabetes Research Portfolio

Type 1 diabetes research at the NIH is supported by regularly appropriated funds that the Department of Health and Human Services (HHS) receives for diabetes research through the Labor-HHS-Education Appropriations Committees. It is also supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*, a special appropriation that the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) administers on behalf of the Secretary and in collaboration with multiple NIH institutes and centers, and the Centers for Disease Control and Prevention (CDC).

The *Special Funding Program* has allowed the creation of unique, collaborative, and innovative research consortia and clinical trials networks to increase understanding about the prevention, treatment, and cure of type 1 diabetes. Initiatives supported by the program are different in size, scope, duration, and nature from other type 1 diabetes efforts supported through regular NIH appropriations. In fact, most of these large-scale, high-impact research efforts could not

otherwise have been undertaken at all, or not at a scientifically optimal scale of operation, without support from the *Special Funding Program*. Importantly, the research efforts that have been supported to date have spurred numerous future opportunities which could dramatically improve the lives of patients with type 1 diabetes.

Collaborative Planning Process

The NIDDK is the lead institute at the NIH for pursuing type 1 diabetes research. Because this research involves diverse scientific disciplines, the NIDDK collaborates extensively with other NIH institutes and centers, as well as other government agencies. Type 1 diabetes research involves nearly every institute and center of the NIH, including the National Center for Research Resources (NCRR), National Eye Institute (NEI), National Human Genome Research Institute (NHGRI), National Heart, Lung, and Blood Institute (NHLBI), National Institute of Allergy and Infectious Diseases (NIAID), National Institute of Biomedical Imaging and Bioengineering (NIBIB), National Institute of Child Health and Human Development (NICHD), National Institute of Dental and Craniofacial Research (NIDCR), National Institute of Environmental Health Sciences (NIEHS), National Institute of Neurological Disorders and Stroke (NINDS), National Institute of Nursing Research (NINR), National Library of Medicine (NLM), and other NIH institutes and centers that are represented on the statutory Diabetes Mellitus Interagency Coordinating Committee (DMICC). The NIH also works closely with the CDC, the Food and Drug Administration (FDA), the Centers for Medicare and Medicaid Services (CMS), and other governmental agencies represented on the DMICC. Also contributing to program planning are the two major diabetes voluntary organizations, the Juvenile Diabetes Research Foundation International (JDRF) and the American Diabetes Association (ADA).

Highlights of Current NIH Funding of Type 1 Diabetes Research

The NIH vigorously pursues and supports research on the understanding, prevention, and cure of type 1 diabetes. Because this research spans such a broad range of disciplines, current efforts are under way in diverse areas, such as genetics, genomics, proteomics, immunology, developmental biology, imaging, bioengineering, glucose sensing, and insulin delivery. Clinical trials test promising agents for type 1 diabetes and its complications. The following are highlights of some of the major efforts and innovative approaches that are currently under way (listed alphabetically). However, these research efforts are illustrative examples and not a comprehensive list of the entire NIH type 1 diabetes research portfolio. The NIH supports investigator-initiated research projects and fosters development of research efforts in areas of particular importance and opportunity through solicitations for grant applications and research contract proposals, and the NIH will continue to strongly support these efforts.

Animal Models of Diabetic Complications Consortium (AMDCC): The AMDCC is an inter-disciplinary consortium designed to develop animal models that closely mimic the human complications of diabetes for the purpose of studying disease pathogenesis, prevention, and treatment. The Consortium has already developed a number of promising models for complications involving the heart, kidney, and nervous system. Development of animal models is essential for pre-clinical drug development.

Beta Cell Biology Consortium (BCBC): The mission of this Consortium is to facilitate interdisciplinary approaches that will advance understanding of pancreatic islet cell development

and function. The long-term scientific goal is to develop a cell-based therapy to restore normal insulin production and action to diabetic patients. Working towards this goal, the BCBC has created and distributed important reagents that will serve the scientific community-at-large. Research pursued through the BCBC can ultimately help to overcome a major barrier to islet transplantation--the shortage of islets.

Clinical Islet Transplantation Consortium (CIT): The purpose of this consortium is to develop and implement a program of single- and/or multi-center clinical studies, accompanied by mechanistic studies, in islet transplantation with or without accompanying kidney transplantation, for the treatment of type 1 diabetes. Research pursued through this consortium aims to make improvements in the field of islet transplantation and share the data and results with the broad scientific community.

Collaborative Islet Transplant Registry (CITR): The mission of the CITR is to expedite progress and promote safety in islet transplantation through the collection, analysis, and communication of comprehensive, current data on all islet transplants performed in North America. The CITR prepares an annual report with data on recipient and donor characteristics, pancreas procurement and islet processing, immunosuppressive medications, function of the donated islets, patients' lab results, and adverse events. This information will help to define the overall risks and benefits of islet transplantation as a treatment option for type 1 diabetes patients.

Cooperative Study Group for Autoimmune Disease Prevention (Prevention Centers): The mission of the Prevention Centers is to engage in scientific discovery that significantly advances knowledge about the prevention and regulation of autoimmune disease, including type 1 diabetes. Pre-clinical research conducted by the Prevention Centers is key to the development of strategies for modulating the immune system so that they can be tested in human clinical trials.

Diabetes Research in Children Network (DirecNet): The focus of DirecNet is to investigate the use of technologic advances in the management of type 1 diabetes in children and to develop a better understanding of hypoglycemia. Goals of the network include assessing the accuracy, efficacy, and effectiveness of continuous glucose monitoring in children with type 1 diabetes, and determining the extent to which exercise contributes to the risk of hypoglycemia. Until cell replacement therapy is a viable treatment option for children with type 1 diabetes, research on glucose sensing and insulin delivery is crucial to improving quality-of-life and decreasing the number of hypoglycemic episodes.

Diabetic Retinopathy Clinical Research Network (DRCR.net): Type 1 diabetes causes damage to the eyes and may lead to blindness. The DRCR.net conducts multi-center clinical research studies to test promising therapeutic agents for the treatment of two forms of diabetic eye disease--diabetic retinopathy and diabetic macular edema--and associated conditions. Because blindness is such a severe and debilitating disease complication, research pursued through this network could dramatically improve patients' quality-of-life.

Epidemiology of Diabetes Interventions and Complications (EDIC): The aim of EDIC is to study the clinical course and risk factors associated with the long-term complications of type 1 diabetes, using the cohort of the Diabetes Control and Complications Trial (DCCT). The

DCCT/EDIC research group has observed dramatic long-term benefits of intensive glucose control in preventing and delaying complications of the eyes, kidneys, nerves, and heart. These results have had a major impact on the clinical care of diabetes patients.

Family Investigation of Nephropathy and Diabetes (FIND): The FIND consortium is carrying out studies to elucidate the genetic susceptibility to kidney disease in patients with diabetes, as well as genetic susceptibility to retinopathy in diabetic patients. A family-based study recruited over 2,500 affected and discordant pairs of siblings. A separate case control study is completing recruitment of over 3,000 individuals. These studies will help researchers understand the genetic underpinnings of the kidney and eye complications of diabetes, which can, in turn, inform prevention and treatment strategies.

Genetics of Kidneys in Diabetes Study (GoKinD): GoKinD was established to study the genetics of kidney disease in type 1 diabetes patients. The study group has collected and is distributing DNA and other biological samples from over 1,700 adults with type 1 diabetes in the U.S. and Canada. Scientists will use these samples to identify genes that are important in the development of, or resistance to, diabetic kidney disease.

Immune Tolerance Network (ITN): "Immune tolerance" is the process by which the immune system accepts a protein or other molecule as "self" and does not attempt to destroy cells or tissues containing that protein. Tolerance induction can block the autoimmune process underlying type 1 diabetes, or enable the body to "accept" transplanted islets without the need to globally suppress the immune system. Research conducted through the Immune Tolerance Network (ITN) is evaluating new treatments to induce tolerance in type 1 diabetes, as well as other disease areas. ITN is currently conducting and developing several clinical trials related to type 1 diabetes and islet transplantation. This type of research is critical to developing promising new strategies to cure type 1 diabetes by islet transplantation.

Islet Cell Resource Centers (ICRs): The Islet Cell Resource Centers (ICRs) serve as regional centers that provide clinical grade human islets to investigators engaged in islet transplantation protocols throughout the country; optimize the procedures used to obtain such islets; and distribute human pancreatic islets to investigators for use in laboratory-based research studies. This type of resource provides high-quality islets for use in human islet transplantation research, and allows researchers to continue to investigate islets in basic research studies.

Non-human Primate Transplantation Tolerance Cooperative Study Group (NHPCSG): This multi-institution Study Group was established to evaluate the safety and efficacy of novel therapies to induce immune tolerance in non-human primate models of kidney and islet transplantation. The program also supports research to understand the underlying molecular mechanisms of immune tolerance. Pre-clinical research conducted by this Group will help scientists move promising therapeutic agents from the laboratory into human clinical trials.

Research Training and Career Development in Pediatric Diabetes: This program provides support of research training and career development in pediatric diabetes at institutions that have environments, mentors, and programs that will make them particularly effective in enhancing the number of independent investigators contributing to research in pediatric diabetes. The awards,

through the T32 (institutional research training) and K12 (clinical scientist career development program) grant mechanisms of the NIH, are intended to provide an opportunity for continuous training from the clinical fellowship years to emergence as a fully trained independent investigator. These integrated programs are designed to prepare pediatricians, selected by the institution, for careers in pediatric endocrinology research related to diabetes.

Search for Diabetes in Youth (SEARCH): There are no comprehensive population-based estimates of diabetes burden among American youth. SEARCH will define the prevalence and incidence of diabetes in children and youth less than 20 years of age in six geographically dispersed populations that encompass the ethnic diversity of the U.S. This study will help increase understanding of how type 1 diabetes strikes and unfolds.

Standardization Programs: Standardized assessment of key measures for type 1 diabetes research is extremely important to ensure consistency across laboratories and clinical trial networks, so that data can be compared and combined. Efforts are ongoing to improve and standardize the measurement of autoantibodies (used to identify initiation of autoimmunity), C-peptide (a measure of beta cell mass and function), and HbA1c (a measure of long-term blood glucose control).

The Environmental Determinants of Diabetes in the Young (TEDDY): The goal of TEDDY is to identify environmental causes of type 1 diabetes in genetically susceptible individuals. The study is enrolling at-risk newborns and then following them until they are 15 years of age. This long-term study is crucial to helping researchers understand the environment triggers that play a role in type 1 diabetes disease onset and development.

Trial To Reduce IDDM in the Genetically At Risk (TRIGR): This multi-center, international study is comparing the development of type 1 diabetes in genetically-susceptible infants who are weaned onto a hydrolysate of cow's milk formula, in which many of the cow proteins have been broken down, *versus* standard cow's milk formula. TRIGR, which is currently in the patient recruitment phase, could have a major impact on disease prevention if differences are observed between the two types of formulas.

Type 1 Diabetes Genetics Consortium (T1DGC): T1DGC is organizing international efforts to identify genes that determine an individual's risk of developing type 1 diabetes. This consortium is currently recruiting 2,800 family members who have two or more siblings with type 1 diabetes in order to identify genes that increase susceptibility. Finding these genes will not only increase understanding of the underlying molecular mechanisms of disease development, but also aid in the discovery of novel prevention strategies, and also help to identify patients who could benefit from these approaches.

Type 1 Diabetes-Rapid Access To Intervention Development (T1D-RAID): The T1D-RAID program provides resources for manufacture and pre-clinical development of drugs, natural products, and biologics that will be tested in type 1 diabetes clinical trials. The goal of T1D-RAID is to facilitate the translation of promising therapeutic agents from the bench to the bedside, in order to more rapidly impact patients' health.

Type 1 Diabetes TrialNet: TrialNet is an international network of investigators, clinical centers, and core support facilities. It supports the development and implementation of clinical trials of agents to slow the progression of type 1 diabetes in new-onset patients and to prevent the disease in at-risk patients. TrialNet has launched several studies that are recruiting patients, and is in the process of evaluating several other therapeutic agents to test in the network. This type of collaborative network infrastructure is critical for facilitating clinical trials in type 1 diabetes, as well as for making real improvements in patients' health by identifying new therapeutic agents.

For more information on these and other type 1 diabetes research efforts, please visit a website dedicated to research supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*: www.T1Diabetes.nih.gov

Development of the Strategic Plan

Origin and Purpose of Plan

In January 2005, the NIDDK convened an *ad hoc* planning and evaluation meeting of external scientific and lay experts in type 1 diabetes to perform a mid-course assessment of many currently funded type 1 diabetes research programs, and to identify future research opportunities within this context. The focus of this meeting was on research consortia and clinical trials networks supported by the *Special Statutory Funding Program for Type 1 Diabetes Research*. A detailed summary of the meeting can be accessed on the NIDDK website at: www.niddk.nih.gov/federal/planning/Jan-18-19-T1D-FINAL.pdf.

One of the recommendations emanating from this meeting was to initiate a much broader review of the entire state-of-the-science with respect to type 1 diabetes, including recent research advances and emerging opportunities. Such a review would be far more encompassing and future-oriented than the assessment performed at the January 2005 meeting, which was largely focused on existing programs. In response to this recommendation, the NIDDK Director announced in March 2005 that the Institute would spearhead a new strategic planning effort in type 1 diabetes research under the auspices of the statutory Diabetes Mellitus Interagency Coordinating Committee (DMICC), chaired by the NIDDK. The membership of this Committee includes all NIH components involved in diabetes research, as well as other relevant federal agencies.

The purpose of this Strategic Plan is to serve as a scientific guidepost to the NIH and to the investigative and lay community by identifying compelling research opportunities. These scientific opportunities will inform the priority-setting process for the type 1 diabetes research field and propel research progress on the understanding, prevention, treatment, and cure of type 1 diabetes and its complications.

Collaborative Planning Process

The Strategic Plan was developed with broad input from a diverse and talented group of researchers and lay experts dedicated to advancing type 1 diabetes research (please see Appendix

A). Participants included representatives from the NIH and other Federal agencies represented on the DMICC, scientists external to the NIH, lay persons representing patients' interests, and representatives from diabetes voluntary organizations.

The Strategic Plan was organized around the six overarching goals of type 1 diabetes research described previously. To formulate the Plan, Working Groups were convened to address each of the first five goals. The sixth goal, "Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes," is an overarching goal that is relevant to all of type 1 diabetes research. Therefore, this goal was addressed by each of the five Working Groups. Each Working Group was chaired by a scientist external to the NIH and was comprised of other external scientific experts, a lay representative, a representative of a diabetes voluntary organization, at least one member of the DMICC, and other senior scientific government representatives. The Working Group members were asked to survey the state of the science and develop a summary of progress and opportunities relevant to each goal.

In addition to the Working Groups, the Strategic Plan was informed by insights provided by an overarching Executive Committee comprised of the chairs of the five Working Groups and representatives from the government and from diabetes voluntary organizations. The Executive Committee met on September 28, 2005, to assure that in aggregate the components developed by the Working Groups were comprehensive and addressed the most compelling opportunities for prevention, therapy, and cure of type 1 diabetes and its complications. They provided guidance on integration of the products of each Working Group into a final Strategic Plan that will serve the purpose of informing future priority-setting in type 1 diabetes research.

Public Input To Inform the Planning Process

To solicit broad public input into the strategic planning process, draft chapters were posted on the NIDDK's website dedicated to type 1 diabetes research (accessed at: www.T1Diabetes.nih.gov).

Organization of the Plan

This Plan has been developed for the scientific research community and for type 1 diabetes patients and their family members. In this regard, the Plan outlines specific research directions that can be pursued to achieve the overarching goals of type 1 diabetes research, and it also describes how achieving the goals will directly benefit the health and quality-of-life of type 1 diabetes patients and their family members.

The scientific chapters have been organized around the six overarching goals of type 1 diabetes research:

- Goal I: Identify the Genetic and Environmental Causes of Type 1 Diabetes
- Goal II: Prevent or Reverse Type 1 Diabetes
- Goal III: Develop Cell Replacement Therapy
- Goal IV: Prevent or Reduce Hypoglycemia
- Goal V: Prevent or Reduce the Complications of Type 1 Diabetes
- Goal VI: Attract New Talent and Apply New Technologies to Research on Type 1 Diabetes

Each chapter is divided into the following sections:

Why This Goal Is Important to People: This section, written in language for the general population, highlights the clinical relevance of the goal, and describes how research progress can have a direct and dramatic impact on the lives of type 1 diabetes patients and their family members.

Strategies to Achieve the Goal: This section includes the following:

- *Introduction and Background*: A brief description of the current state-of-the-science, and an overview of the importance of the goal in propelling research progress in type 1 diabetes.
- *Recent Research Advances*: Examples of major breakthroughs in type 1 diabetes research that have made a significant impact on the research field or patients' health, particularly in the last 5-7 years.
- *Research Objectives and Strategies to Achieve Goals*: The objectives are specific research directions that can be pursued to realize the goal of the chapter. The objectives were identified by Working Group members as being critically important for overcoming current barriers and achieving progress in type 1 diabetes research relative to the chapter's overarching goal over the next 10 years. This section also describes some immediate steps that can be taken to implement the research objectives.

Implementation of the Strategic Plan

Successful implementation of the research objectives outlined in this Plan requires the collaboration of the multiple institutes and centers of the NIH, other government agencies represented on the Diabetes Mellitus Interagency Coordinating Committee, industry, and the diabetes research and voluntary community. It is only through the involvement and collaboration of these different entities that research progress will be realized.

Although this document, which reflects current research advances and objectives, is necessarily "static," the strategic planning process is dynamic. Novel findings and new technologies can dramatically and positively change the course of planned research. Therefore, in order to for this Plan to be successful, it must periodically be assessed by scientific experts in the type 1 diabetes research field so that new and emerging opportunities can be identified. The Diabetes Mellitus Interagency Coordinating Committee will serve an important role by assessing progress toward attaining the goals and objectives described in the Plan. The NIH will also continue to solicit the input of the broad scientific community through forums such as scientific workshops, conferences, and planning and evaluation meetings. This input will continue to be a valuable and necessary component of the NIH's strategic planning process for type 1 diabetes research.

Looking Forward: Future Type 1 Diabetes Research

Research efforts over the past several decades have led to tremendous improvements in the health and quality-of-life of type 1 diabetes patients. The prognosis for newly diagnosed patients has drastically improved compared to just a decade ago. While these improvements are positive, one thing remains certain: "we are not there yet." Persons with type 1 diabetes still check their blood sugar, administer insulin, and develop life-threatening complications. It is imperative to build upon the strong existing research base to not only improve current treatment strategies, but also to identify ways to prevent and cure the disease. Because of new and emerging technologies in areas such as genomics, imaging, and systems biology, there is great potential to make significant and dramatic improvements in the health of type 1 diabetes patients in the near future. Thus, it is important to harness these technologies for type 1 diabetes research and to sustain and intensify the momentum that currently exists in the field. Achieving the specific objectives and making progress toward the overarching research goals outlined in this Strategic Plan will have an enormous impact on type 1 diabetes patients, as well as on patients with other forms of diabetes and other autoimmune diseases.

GOAL I: IDENTIFY THE GENETIC AND ENVIRONMENTAL CAUSES OF TYPE 1 DIABETES

Why This Goal Is Important to People

TYPE 1 DIABETES IS AN INSIDIOUS, DESTRUCTIVE AND COSTLY DISEASE THAT CAN STRIKE ANYONE. THOSE WHO DON'T HAVE THE DISEASE USUALLY KNOW SOMEONE WHO DOES—A FAMILY MEMBER, FRIEND, NEIGHBOR OR CO-WORKER. THEY ASK THEMSELVES: "AM I AT RISK FOR THIS DISEASE TOO, CAN I BE TESTED FOR IT, AND HOW CAN I PREVENT IT FROM STRIKING ME OR MY CHILD? IS THERE ANY WAY TO CHANGE MY DIET OR THAT OF MY CHILDREN TO HELP PREVENT THE DISEASE?" PATIENTS LIVING WITH THE DISEASE ASK: "HOW DID I GET THIS DISEASE? DO I HAVE A BAD FORM OF THE DISEASE? WILL MY CHILDREN OR MY GRANDCHILDREN BE LIKELY TO GET IT TOO?"

Unraveling the Complexities of Type 1 Diabetes

In type 1 diabetes, an interplay of genetic and environmental factors is at the root of the immune system's misguided attack on the body's insulin-producing cells (beta cells found in clusters called "islets" within the pancreas). Until these factors are completely deciphered, it will not be possible to know with certainty all those who are at risk for the disease and their specific risk profiles. This knowledge is urgently needed in order to develop and tailor the most effective clinical strategies for completely preventing the disease. This knowledge would also facilitate research aimed at reversing the disease as soon as possible after onset—before complications take hold of the eyes, kidneys, nerves, heart, and other parts of the body.

Type 1 diabetes is an extremely complex disease believed to involve many genes, which work in concert and can have both large and small effects. If altered from their healthy state, the genes can cause a person to have a predisposition for the disease. When this genetic susceptibility is "triggered" by an environmental agent, the body's immune defense system will then turn against itself. Ironically, when provoked, the normally protective immune system—which fights against bacteria, viruses, and other foreign invaders—will mistakenly launch an assault on the body's own insulin-producing cells. This immune-system attack on "self" makes type 1 diabetes an "autoimmune" disease.

Finding Culprit Genes and Their Role in Autoimmunity

It is important to find out why some individuals develop type 1 diabetes, while others do not. The likelihood that a person will develop the disease is known to be higher the more closely related he or she is to someone who has type 1 diabetes. However, 80 percent of new type 1 diabetes patients do not have close relatives with the disease (14). Moreover, even in identical twins who have the same genetic make-up, it is possible for the disease to affect one, but not the other.

Many research advances have been achieved in the search for "culprit" genes, their variations, and their influence on the immune system. Strong evidence points to four genetic regions that contain suspect genes. However, both laboratory and clinical studies indicate that as many as 20 other regions may contain genes that influence disease susceptibility, and some of these genes may influence it only in certain populations. Moreover, it is possible that greater risk is conferred by specific gene combinations and gene-gene interactions, whereas smaller risk may accompany the presence and interplay of other genes. Teasing apart these differences is extremely difficult.

Research indicates that one of the implicated genetic regions (the major histocompatibility complex or "MHC") may contribute up to 50 percent of the total genetic risk for type 1 diabetes. Moreover, the protein products of genes in this region are of central importance to the body's immune response. It is possible that these gene products "trick" key immune-system cells (T cells) into attacking proteins in the pancreas as if they were invading bacteria or viruses. Other studies have confirmed that some people have a version of the insulin gene that makes them more susceptible to type 1 diabetes. In particular, they have shown that the degree of disease susceptibility is likely to be directly influenced by the number of repeated elements in a region of this gene that regulates its expression. Still other research has revealed genes that dampen or eliminate proteins that protect the body against an aberrant immune response.

To pre-empt or intercept the type 1 disease process, it is essential to learn the exact genes, proteins and immune-system components involved, as well as the panoply and sequence of events. Finding biologic markers of the disease process or ways to image its stages will also further research efforts. Expanding the knowledge base in these and other ways could then open up important research avenues toward multiple means of prevention.

Environmental Factors

In parallel with the search for disease-causing genetic factors, it is imperative to uncover the environmental triggers that spark type 1 diabetes. Many individuals may have a genetic susceptibility to the disease, but may never actually develop it unless something in the environment initiates that genetic machinery. Environmental triggers remain elusive—although research suggests that viruses, diet, environmental toxins, and/or stress may be implicated. Observed patterns of disease outbreak, as well as seasonality of onset, lend support to the possibility that an infectious agent may act as a trigger. The role of dietary factors in protecting against getting the disease has yet to be established. Studies have suggested that vitamins B and D, as well as certain fatty acids, may have protective effects, but more research is needed on the role of these and other dietary factors in disease development. Importantly, the studies of environmental factors that play a role in type 1 diabetes may also contribute to understanding the development of celiac disease, which is an autoimmune disease primarily affecting the gastrointestinal tract. In the U.S., the prevalence of celiac disease has been estimated to be approximately one percent (15). Some genes confer susceptibility to both celiac disease and type 1 diabetes and many people have both diseases. Therefore, ongoing studies to identify environmental triggers of type 1 diabetes are also investigating development of celiac disease. These studies may uncover environmental factors initiating both disorders, benefiting not only type 1 diabetes patients, but also persons suffering from celiac disease.

Long Term Studies

Very long-term studies are required to understand the causes and natural history of type 1 diabetes. These types of studies are needed because environmental triggers of disease often occur in infancy and early childhood, but the disease onset may be later in childhood, adolescence, or early adulthood. Some efforts to this end are already under way in NIH-funded consortia and should be continued. Recently, scientists directing six independent studies of environmental triggers of type 1 diabetes in the U.S. and high-risk areas of Europe joined forces to create a united study (TEDDY) with much greater power to uncover potential environmental triggers. Importantly, samples from the TEDDY study will be made widely available to researchers worldwide. Already, elimination of early exposure to one potential dietary trigger of type 1 diabetes, cow's milk based infant formula, is being tested in a clinical trial (TRIGR). While it is a substantial investment of time and resources to follow individuals for many years, such long-term studies could answer critically important questions about disease risk and onset. For example, if a viral trigger is revealed, then a vaccine could possibly be developed to prevent disease onset in genetically susceptible individuals.

Consortia for Pooling of Genetic Resources and Talent

Type 1 diabetes research benefits greatly from generic, large-scale projects, such as the Human Genome Project, which have accelerated the study of genes and their function in health and disease. These types of broad efforts provide a knowledge base that can be greatly amplified by the addition of disease-specific genetic data, such as that being garnered by the ongoing NIH-funded international Type 1 Diabetes Genetics Consortium. Because this Consortium is specific to type 1 diabetes, it is a powerful tool for unraveling the complex underpinnings of the disease. The search for type 1 diabetes genes will be furthered by studies with extensive collections of disease-specific genetic information and biomaterials, and the combined expertise of many investigators. Analyses of large study groups offer the statistical power needed to identify and confirm genetic and environmental contributors to complex diseases. Such pooled resources increase the probability of not only defining genetic risk, but also of identifying targets toward which new preventive strategies can be directed.

Pursuit of Candidate Genes and Insights from Animal Models

To narrow the gene-hunt, researchers have identified and are continuing to focus their efforts on genes believed to be likely "candidates" for contributing to disease onset. Many additional candidate genes will be identified by general immunology studies, research on insulin-producing cells, and investigations of animal models that mirror type 1 diabetes in humans. Discovery of diabetes-causing genes in animal models will propel research on corresponding genes in human tissue samples, and will thus help to uncover the pathways in which the genes function. Every "culprit" gene and pathway that is identified represents a potential target for heading off the disease before its onslaught, or for intervening in the disease before it progresses to serious complications.

Future Implications of This Research

With new insights into the interplay of genetic-environmental factors and immune mechanisms in type 1 diabetes, researchers may be able to identify with great precision those individuals at risk for the disease, and to develop and test prevention-oriented strategies. It is possible, for example, that such new knowledge could point the way toward the screening of newborns, and to even more widespread screening to identify individuals at risk in the general population. This knowledge would facilitate the design of more specific clinical trials for testing interventions specifically tailored to patients with similar risk profiles. If researchers find that an infectious agent is an environmental trigger of the disease, efforts could be directed toward the development of a preventive vaccine. Alternatively, if a dietary component is found to be causative or protective, individuals at risk could take steps to either eliminate or add it to their diets. These are just a few examples of the enormously important and practical strides forward that can be envisioned and possibly attained once the underlying causes of type 1 diabetes are fully delineated.

Diabetes is an extremely costly disease to treat in both human and financial terms; it places an enormous burden on families and on the U.S. health care system. By pinpointing the constellation of type 1 diabetes disease genes, their environmental triggers, and their cascading effects on the immune system, researchers may be able to entirely prevent or reverse disease onset. Combating the disease at the “front-end” is especially beneficial because early steps could preclude or arrest the development of disease complications—including kidney failure, blindness, lower limb amputations, heart attacks and strokes. Research on the underpinnings of the disease thus offers the real hope of preventing type 1 diabetes from ever ravaging the body. For individuals at risk, it would clearly be far better to completely prevent the disease than to undergo a difficult and suboptimal treatment regimen of daily insulin administration after the disease has begun wreaking havoc with the body. Likewise, the Nation as a whole would benefit from building a sound knowledge base for developing prevention-oriented strategies.

GOAL I: IDENTIFY THE GENETIC AND ENVIRONMENTAL CAUSES OF TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

Type 1 diabetes is characterized by autoimmune destruction of insulin-producing pancreatic beta cells. It has long been known that the likelihood of a person developing type 1 diabetes is higher the more closely related he or she is to a person with the disease. However, even in monozygotic ("identical") twins, this probability is much less than 100 percent (16), and indeed 80 percent of new type 1 diabetes patients do not have close relatives with the disease (14). The disease also exhibits patterns of outbreaks and seasonality consistent with involvement of infectious agents. These observations suggest that, in addition to a strong genetic component, an environmental factor or factors may also play a role in causing type 1 diabetes.

Type 1 diabetes risk is influenced by multiple genes. To date, there is strong evidence that four regions of the genome are known to contain genes related to type 1 diabetes: the major histocompatibility complex (MHC) that includes genes that encode the Human Leukocyte Antigens (HLA) on chromosome 6; the region around the insulin gene (*INS*) on chromosome 11; a region that contains several immune response genes (including *CTLA4*) on chromosome 2; and the protein tyrosine phosphatase N22 *(PTPN22)* gene on chromosome 1. Studies in both mouse and man indicate there may be as many as 20 other regions containing genes that influence susceptibility to type 1 diabetes. Some of the genes may influence disease only in some populations. In other cases, multiple genes could interact such that the risk associated with specific gene combinations is great, while the risk associated with any one of the genes alone could be small. These factors make identifying the responsible genes challenging.

The environmental contributors to type 1 diabetes are also likely to be complex. A variety of triggers have been suggested. These include viruses, diet, environmental toxins and stress. However, no definitive proof of a causative link with any of these factors has yet been found. Understanding, preventing, and treating type 1 diabetes critically depends on greater understanding of its causes. The *Special Statutory Funding Program for Type 1 Diabetes Research* has enabled several large-scale clinical studies that will facilitate further understanding of type 1 diabetes genetics, etiology, and prevention.

RECENT SCIENTIFIC ADVANCES

The identification of genes and genetic regions contributing about half the genetic risk for type 1 diabetes has been key to the successful development of clinical trials to test strategies to prevent type 1 diabetes, and clinical studies to identify environmental triggers. This genetic information has allowed identification of individuals at risk for type 1 diabetes who might benefit from participation in these clinical studies. Subsequent progress in identifying genes with smaller contributions to risk is opening up new areas of investigation into the pathogenesis of type 1 diabetes and potential new strategies for interventions. The confirmed type 1 diabetes susceptibility genes and gene variants are being employed to describe in detail the genetic and

molecular basis for type 1 diabetes pathogenesis in order to identify relevant biological pathways involved as a basis for targeted therapies and drug development.

HLA Genes Contributing to the Risk of Type 1 Diabetes: The genetic basis of type 1 diabetes is complex and likely to be due to genes of both large and small effect and the interaction of these genes. Numerous studies have investigated genetic susceptibility loci, using both case-control and family study designs. Allelic variation (different versions) in two HLA genes in the MHC class II region (HLA-DRB1 and HLA-DQ1) have been shown to represent the primary genetic determinants of risk for type 1 diabetes, although other class II (HLA-DPB1) as well as class I (e.g., HLA-A, HLA-B) and class III (e.g., TNF) genes may contribute to susceptibility. It has been suggested that genes in the MHC may contribute up to 50 percent of the total genetic risk for type 1 diabetes, although the effect of HLA genes likely represents more than simple increase of risk. Products of the MHC class II genes are centrally important in the immune response. These proteins bind short peptides derived from foreign (or self) proteins and "present" them to cells (designated T cells) that coordinate the immune response. If the T cell does not recognize the peptide as coming from a "self" protein, it initiates an immune response. It is also not clear whether or by how much other genes in the region also affect diabetes susceptibility, because the strong effects of the MHC class II genes may overshadow weaker, but still important, contributions of risk by other genes.

The Contribution of *INS* to Type 1 Diabetes Susceptibility: A series of studies has confirmed an association of type 1 diabetes with the insulin gene, *INS*, and in particular that susceptibility to type 1 diabetes is likely to be directly influenced by the number of repeated elements in the *INS* gene, called the "variable number of tandem repeats region," or VNTR. From studies of European and U.S. families, smaller numbers of repeats (designated class I VNTRs) generally confer increased risk for disease. Larger numbers of VNTR repeats, designated class III, confer a degree of protection from disease. Interestingly, although humans get a copy of the *INS* gene from each of their parents, it is only necessary for one of those copies to be class III in order to confer resistance to type 1 diabetes. Since the class III VNTRs are associated with higher levels of insulin mRNA in the thymus, it is possible that lower risk of diabetes is associated with higher thymic expression, and thereby higher rates of deletion of self-reactive T cells during development. This possibility has yet to be proven in humans. Furthermore, data from mice that show that a portion of the insulin precursor protein is essential for type 1 diabetes in the Non-Obese Diabetic (NOD) mouse are consistent with the hypothesis that expression of the insulin precursor can directly affect type 1 diabetes incidence in humans.

Other Genetic Factors Associated with Susceptibility to Type 1 Diabetes: Recent studies revealed that the *PTPN22* and *CTLA4* genes also contribute to several autoimmune diseases, including type 1 diabetes. Both genes encode proteins that act as negative regulators of T-cell activation. Another gene, AIRE, exerts an immune tolerance-promoting function by negative selection of T effector cells in the thymus. The absence of the AIRE protein, which results from a rare mutation in people, promotes autoimmunity in several tissues, and increases the incidence of type 1 diabetes and other autoimmune diseases.

Exploration of Human Genomic Regions Associated with Type 1 Diabetes Susceptibility: While candidate genes for type 1 diabetes are the subject of numerous ongoing investigations,

there has previously been little coordinated effort to fully explore the regions around these candidate genes or the potential interactions between these genes. Regions of the genome in which linkage to, and/or association with, type 1 diabetes include portions of human chromosomes 2q (*IDDM7-IDDM12*, *IDDM13*), 3 (*IDDM9*), 5q (*IDDM18*), 6 (*IDDM15*, *IDDM5*/*TAB2*/*SUMO4*, and *IDDM8*), 10 (*IDDM10* and *IDDM17*), 11q (D11S1296-FGF3), 14 (*IDDM14*) and 16. These regions each have a relatively small impact on type 1 diabetes susceptibility, so that published studies have insufficient statistical power to precisely quantify their influence on type 1 diabetes susceptibility. In order to accurately gauge their impact, large numbers of affected sib-pair families, parent-child trios, or case-control collections will need to be studied.

The Type 1 Diabetes Genetics Consortium (www.t1dgc.org) has provided for collection of biological materials required to conduct in-depth and sufficiently-powered genetic studies. The creation of repositories for DNA, plasma, and serum, for genetic studies provides a resource for research advancement in a cost-effective manner. The ability to discover genes that cause complex diseases has also been greatly facilitated by breakthroughs in large-scale sequencing, genotyping, and data analysis. Continued examination of candidate genes, linkage regions, and application of whole genome association studies, coupled with integration of epidemiologic risk factors identified by other consortia activities, could identify critical pathways that better define an individual's risk of type 1 diabetes.

Genes and Genetic Concepts Discovered in Animal Models of Type 1 Diabetes: The NOD mouse and BB rat models are currently the most studied animal models of human type 1 diabetes. Importantly, genetic variability in the peptide-binding pockets of the products of the MHC class II genes--both in humans and in these animal models--is a major determinant of susceptibility to type 1 diabetes. As in humans, both CD4 and CD8 T lymphocytes invade the pancreas in animal models, and disease development is sensitive to immunosuppression. Insulin autoantibodies appear early in the disease process in the NOD mouse and, as in human type 1 diabetes, anti-insulin antibodies are predictive of disease development. Although the BB rat model also has significant similarities to human type 1 diabetes, the lymphopenia present in this model is essential for the development of type 1 diabetes in most situations, a feature not shared with human type 1 diabetes.

Multiple type 1 diabetes susceptibility regions have been identified in NOD mice. These include *Idd3*, *Idd10*, and *Idd18* on chromosome 3; *Idd5.1*and *Idd5.2* on chromosome 1; and *Idd9.3* on chromosome 4. The regions are orthologous with human 4q28-28.1, 1p12-13.1, 1p13.3, 2q33.2-33.3 [*IDDM12*], 2q35, and 1p36.22-35.31, respectively. Type 1 diabetes regions 10 mega base pairs (Mbp) in the NOD mouse can be sequenced via an NIH-sponsored NOD sequencing initiative to reveal disease-associated polymorphisms that exist between the B6 and NOD strains. Recently described mouse and rat models of type 1 diabetes, such as the ALR mouse and the LEW.1AR1 iddm rat, will add to this knowledge of gene variants conferring protection from type 1 diabetes, particularly via molecular pathways active within the beta cells themselves.

Disease genes identified thus far in the NOD mouse model include functional variants encoding beta 2 microglobulin (within Idd13) and CTLA4 (Idd5.1). Studies on the function of beta 2 microglobulin allotypes in the pathogenesis of type 1 diabetes in the NOD mouse highlight the

important role of CD8 cells, a subset thought to be critical in mediating human type 1 diabetes. The NOD model provides a means to discover the downstream consequences of disease-associated alleles via molecular and cellular studies. Identification of relevant biological pathways is the basis for targeted preventive strategies and drug development. An additional role of the NOD model has been the creation of strains of these mice carrying the human class I and II alleles associated with susceptibility to or protection from autoimmune disease. Similar "transgenic" approaches to study the function of non-MHC disease genes discovered in human genetic studies will take advantage of the similarities of the mouse and human immune systems, including autoimmune disease pathogenesis.

Type 1 diabetes in the BB rat is dependent on genetic variants in several loci, including *Iddm2* on chromosome 4 that is responsible for the lymphopenia phenotype (reduction in the number of lymphocytes) and is essential to diabetes. The lymphopenia was demonstrated to be due to a mutation in a novel member (*Gimap5*) of the gene family encoding GTPases of the immunity–associated protein family termed GIMAP that results in truncation of a significant portion of the protein. The GIMAP gene family--lying within a tight cluster on rat chromosome 4, mouse chromosome 6, and human chromosome 7--is poorly characterized, but appears to be involved in the control of cell survival.

Perhaps equally important as the efforts to identify genes and genetic pathways affecting diabetes development in various animal models are the genetic insights that have been made from the analysis of mammalian species having an autoimmune disease process influenced by numerous genes. Although studies in non-mammals have contributed significantly to the understanding of genetic interactions, the existing mouse and rat models of type 1 diabetes allow investigators to design experiments to measure downstream molecular and cellular consequences of specific gene combinations. Lessons learned from such experiments are critical for improving the modeling of human data in order to reveal gene-gene interactions. The development of additional animal models with greater fidelity to type 1 diabetes will be critically important to identify new targets for therapeutic interventions.

Initiation of Studies To Identify Environmental Causes of Type 1 Diabetes: Recent evidence suggests that the incidence of type 1 diabetes in children in the U.S. and several other countries has increased over the last 20 years, increasing particularly in young children and infants. The SEARCH consortium, funded by the CDC and NIDDK, is assessing the incidence and prevalence of all forms of diabetes in youth and will contribute to increased understanding of the pathogenesis of the disease. Six clinical centers located in California, Colorado, Hawaii, Ohio, South Carolina, and Washington State will examine approximately 9,000 diabetic children to determine the etiology of their disease, including genetic and environmental determinants. Nevertheless, the causes underlying the increasing incidence rate in the U.S. are not well understood. Diabetes registries may be helpful for this effort. For example, U.S. and state health departments may elect to officially designate type 1 diabetes as a reportable disease, as has been done in Oregon. Data from children and youth developing diabetes will provide more information to better understand the different types of diabetes currently affecting U.S. children.

Numerous studies have investigated the environmental causes of type 1 diabetes, but have not yielded consistent results. This may be due in part to the failures to account for genetic

susceptibility, begin observation at an early age or *in utero*, or monitor patients frequently and long-term. The Environmental Determinants of Diabetes in the Young (TEDDY) consortium has developed a comprehensive multidisciplinary and rigorous approach to this problem. Researchers in the consortium are establishing a cohort of children with elevated genetic risk for type 1 diabetes by screening newborns in the general population and in families with first-degree relatives diagnosed with type 1 diabetes. This research will lead to better understanding of disease pathogenesis, which provides a foundation for new strategies to prevent, delay, or reverse type 1 diabetes. Although the National Children's Study (NCS) is also studying the effects of environmental influences on the health and development of more than 100,000 children across the U.S., it is not powered to achieve statistically significant results for type 1 diabetes. Therefore, the TEDDY consortium is a unique and necessary effort to identify environmental triggers of type 1 diabetes.

Role of Diet in Type 1 Diabetes: Dietary manipulations in rodent models of type 1 diabetes (BB rat and NOD mouse) affect spontaneous diabetes development. Large-scale epidemiologic studies have also suggested that early introduction of cereal into the diet may increase the risk of type 1 diabetes. The TEDDY consortium will seek to validate these preliminary findings. A large international clinical trial, the Trial to Reduce IDDM in the Genetically at Risk (TRIGR), is under way to definitively answer whether early infant exposure to cow's milk increases risk of type 1 diabetes.

Other factors, such as vitamins B and D, have long been known to modify type 1 diabetes risk in the NOD mouse and BB rat. Epidemiologic studies indicate that children are less likely to develop type 1 diabetes when cod liver oil is consumed by the mother in pregnancy, by the infant in the first year of life, or both. A recently initiated feasibility study being performed by the TrialNet consortium, the Nutritional Intervention to Prevent (NIP) type 1 diabetes, is a pilot study of N-3 fatty acid, docosahexaenoic acid (omega-3 fatty acid), given to pregnant mothers, as well as to newborns at risk before 6 months of age.

Obesity may be a risk modifier for type 1 diabetes. *GAD2* has recently been identified as a strong candidate gene for obesity in certain ethnic populations. *GAD2* is expressed in pancreatic beta cells, and genetic markers called "single nucleotide polymorphisms" (SNPs) in *GAD2* that are associated with obesity also modulate insulin secretion. HLA may have an effect on birth weight, and rapid early growth may increase the risk for type 1 diabetes. Studies in rats suggest that manipulation of the intrauterine milieu by calorie restriction or by low protein diet given throughout gestation affects gene expression in islets and other tissues that may be relevant for beta cell sensitivity to cytokines and toxins in the offspring. Variation within *GAD2* and other genetic factors may be important for the development of islet autoimmunity. These results merit follow-up in longitudinal studies in humans.

Role of Viruses in Type 1 Diabetes: Prospective studies of young children at high-risk of developing type 1 diabetes suggest that early and repeated exposure to enteroviruses (e.g., Coxsackie viruses) may trigger autoimmunity leading to type 1 diabetes, and that genetically-determined host responses to virus infection may influence susceptibility to the disease. It has been proposed that historical improvements in hygiene may have resulted in decreased immunity to human enteroviruses. The increasing rate of type 1 diabetes in children could therefore be the

result of the decrease in humoral protection from enteroviral infections that pregnant women can transfer to their fetuses and mothers can transfer to breast-fed children. For the first time, the TEDDY consortium will test this hypothesis in a standard, prospective manner for enteroviruses as well as other viruses, in several key populations. While large epidemiologic studies have ruled out changes in routine childhood immunizations as a cause of type 1 diabetes, studies such as TEDDY will monitor the effect of changes in immunization program on the risk of autoimmune disease.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

Key objectives for research on type 1 diabetes are to develop means of genetic and environmental risk detection through targeted screening and diagnosis, and to use the identified risk factors to develop interventions to block development of the disease. Research on these mechanisms will provide new insights not only for the risk of type 1 diabetes, but also for the development, diagnosis, and treatment of other autoimmune diseases, such as thyroid disease, celiac disease, Crohn's disease, rheumatoid arthritis, lupus, and multiple sclerosis. Research on the genes and variants responsible for susceptibility to type 1 diabetes is progressing on several parallel tracks, facilitated by advances in genetic technology and characterization of the underlying autoimmune phenotypes and environmental risk factors.

Although there has been tremendous progress in genetic and epidemiologic studies of type 1 diabetes, the underlying pathogenesis of the disease and its autoimmune process remain unresolved (as discussed in Goal II). Further research is warranted to identify regions of the genome that harbor type 1 diabetes susceptibility genes, elucidate the genes and their disease-promoting variants, understand the mechanistic functions of the variants, and clarify their interaction with other genes and environmental risk factors and triggers. These research efforts will be important for identification of therapeutic targets and the implementation of molecular medicine strategies for prevention of disease.

The development of several consortia to focus on specific areas of research has been highly productive. Continued progress in understanding the pathogenesis of type 1 diabetes requires collaboration and coordination among geneticists, immunologists, epidemiologists, and experts in infectious diseases and nutrition, both across biomedical research sites and federal and private funding agencies. Hence, further coordination among the consortia, as well as continued support of *ad hoc* working groups, could significantly enhance communication and collaboration.

Many studies, such as T1DGC, TEDDY, the TrialNet Natural History Study, SEARCH and TRIGR, are accumulating substantial amounts of data and samples that can be used to better define genotypes and phenotypes in patients with type 1 diabetes and their family members. Targeted funding mechanisms would encourage novel applications of genomics, proteomics and metabolomics to utilize these resources for exploration of aberrant function of genes, proteins and metabolites for risk of type 1 diabetes.

Genetic Causes

Previous constraints to understanding the genetic basis of type 1 diabetes have been the limited number of samples available for analysis, the lack of molecular genetic reagents available to pinpoint susceptibility loci within the human genome, the limitations of analytic and informatic infrastructure available to understand the genetic data, and the inability to functionally characterize potential causative variants in appropriate model systems. At present, progress in several of these areas provides new opportunities. The T1DGC has initiated collection of renewable genetic materials for use in family-based linkage and association studies. While these resources will be available and will represent an important resource for research, even greater sample sizes will be required for populations with low prevalence of type 1 diabetes and for cohort, case-control, and trio collections. Expanding sample collection and establishing renewable and available collections for research requires continued support. It is anticipated that many genes will be identified that are associated with type 1 diabetes and autoimmunity. Because various ethnic groups have different genetic risk factors for type 1 diabetes, pursuit of the genetic basis for these causal variants in multiple populations remains a high priority.

The development of genomic technology continues at a rapid pace. The reagents of the Human Genome Project and the HapMap project will permit detailed interrogation of candidate regions and genes that may modulate risk of type 1 diabetes. At present, the resources required for initiation of a large-scale, intensive evaluation of candidate regions and genes are available only at a few institutions. The mechanism of epigenetics also needs to be investigated. Further support for genomic capabilities dedicated to type 1 diabetes would permit more comprehensive research to be performed by more investigators. The analyses and management of genomic data remain major obstacles to pursuing type 1 diabetes susceptibility genes. An increased focus on research training and on providing support for the development of cost-effective human DNA sequencing methods and infrastructure for gene-gene and gene-environment analyses should be supported.

Candidate gene studies of type 1 diabetes susceptibility will continue to be informative using both family and case-control designs. Many candidate genes will be identified by general immunology studies, by research on beta cells, and by investigations of animal models of type 1 diabetes, such as the NOD mouse and the BB rat. The effort to discover additional causative genes in animal models of type 1 diabetes will suggest investigation of the corresponding genes directly in human samples--such as those of the T1DGC--in order to provide insights on the pathways in which the causative genes function.

Research Objective—Create Resources for the Study of Type 1 Diabetes Genetics:

- **Complete the Type 1 Diabetes Genetics Consortium (T1DGC), an unlimited source of DNA for type 1 diabetes gene discovery from informative families representing various ethnic groups.**

The T1DGC is establishing a resource of biological materials that will facilitate research on the genetic basis of type 1 diabetes. In addition, the T1DGC will refine the regions in the genome that contain both MHC and non-MHC type 1 diabetes susceptibility genes using high-throughput linkage disequilibrium mapping methods. The T1DGC will complete a genome-wide scan with power to detect susceptibility loci with low locus-specific odds

ratios, evaluate evidence for gene-gene interactions, and clarify, using appropriately large and statistically powered samples, the effects of hypothesized type 1 diabetes susceptibility loci (e.g., *IL12B, SUMO4*) on disease risk.

- **Establish a resource of biological materials that will facilitate research on the genetic basis of type 1 diabetes in those who develop the disease later in life.**

Type 1 diabetes is not only a disease of young age groups. In fact, up to 50 percent of classical type 1 diabetes (carrying HLA risk alleles and islet cell antibody) may occur after the age of 35 (17). Little information is available about the genetic and environmental causes of type 1 diabetes in patients outside the pediatric population.

Research Objective—Identify Human Genes Causing Type 1 Diabetes Using the Candidate Gene Approach:

- **Complete a systematic search for type 1 diabetes susceptibility genes in the MHC region, to identify the mechanisms by which the genes within the human MHC contribute to the major genetic susceptibility in type 1 diabetes, and to estimate the influence of HLA on other genes with respect to type 1 diabetes risk.**

Genes encoding HLA in the MHC region (described previously) are recognized to be the major genetic risk factors for type 1 diabetes susceptibility, because they account for nearly 50 percent of the genetic risk. Yet the type 1 diabetes susceptibility genes in the MHC have not been fully identified or characterized. Mapping and identification of other loci within the MHC region in Caucasian populations is limited by the extensive linkage disequilibrium in the region and the consequently limited haplotype diversity. In other ethnic groups, the prevalence of type 1 diabetes is much lower, and both disease-associated alleles and patterns of linkage disequilibrium within these populations differ from those observed in Caucasians. These populations offer several significant advantages for mapping and identifying risk variants within the HLA-encoding region, for quantitation of allele-specific degree of risk, and for completion of fine mapping, both in the HLA portion and in other regions of the MHC where significant evidence of linkage exists.

- **Identify and elucidate the mechanism of non-MHC type 1 diabetes susceptibility loci, and develop, test, and validate appropriate statistical methods for characterizing genome-wide gene-gene interactions.**

Combined, genes other than those in the MHC account for 50 percent or more of the genetic risk for development of type 1 diabetes; however, these genes are likely to have smaller individual effects, and may interact with other genes. Thus, there is a need to elucidate the mechanisms whereby the relatively minor (non-MHC) type 1 diabetes susceptibility loci (e.g., *CTLA4* and *PTPN22*) modulate risk of disease. Several loci of relevance may escape identification in linkage analyses of type 1 diabetes families or may be found only in extremely large association studies (e.g., *PTPN22*). Although contributing comparatively minor degrees of risk, such genes could be important as potential drug targets. More such genes may be identified in gene-gene interaction studies and may require the development of new molecular and analytic methods for their discovery. Clusters of interacting genes may facilitate identification of cellular pathways involved in type 1 diabetes pathogenesis and may explain the recognized clinical heterogeneity in disease presentation (differences in age at onset, presence of autoantibodies, and risk for organ-specific complications).

- **Utilize newly developed genomic resources to facilitate testing and cataloging of genomic architecture (SNPs and haplotype blocks) to discover all genes and gene variants affecting susceptibility to type 1 diabetes through a genome-wide association study.**

The Human Genome Project has provided researchers access to the complete sequence of the human genome, greatly facilitating the ability to study the genetics of human diseases. New activities of the Human Genome Project include re-sequencing genes, SNP discovery, and the HapMap project, enabling new disease-specific projects. Development of DNA sequencing at the individual level will provide genomic data at an enlarged scale previously unimagined; however, there will need to be improved informatic and analytic procedures developed to understand these sequence data in light of disease susceptibility.

- **Test in prospective clinical studies which genetic factors affect the development of islet autoimmunity, progression to type 1 diabetes, or both.**

Type 1 diabetes is best predicted by the presence of islet autoantibodies. The majority of autoantibodies are directed against insulin, glutamic acid decarboxylase (GAD65) and IA-2. Autoantibodies against one or several of these autoantigens indicate the presence of islet autoimmunity. Some but not all individuals with islet autoimmunity may go on to develop type 1 diabetes. Ongoing prospective studies such as TEDDY, TRIGR, and the TrialNet Natural History Study offer the opportunity to clarify whether genetic factors influence the progression from autoimmunity to type 1 diabetes. Long-term follow-up of these valuable cohorts will be required to address this important question.

Research Objective—Use Knowledge About the Genetic Underpinnings of Type 1 Diabetes To Prevent and Treat the Disease:

- **Integrate knowledge of genetic susceptibility into risk assessment targeted at prevention and treatment of type 1 diabetes.**

Pinpointing those at risk for type 1 diabetes is an essential pre-requisite for clinical trials and implementation in the general population of validated preventative approaches. Approximately 90 percent of all type 1 diabetes patients have either the DRB1*03,DQB1*0201 or the DRB1*04,DQB1*0302 haplotype, but many with these genotypes will not develop diabetes. At present, only HLA is used for genetic screening of patients for type 1 diabetes risk. With the knowledge of involvement of HLA and non-HLA genes in susceptibility to type 1 diabetes, one can combine HLA with non-HLA diabetes risk genes to better define the type 1 diabetes risk levels. This information should be incorporated into the design of prospective studies such as TEDDY and TRIGR and for evaluation for individual risk prediction of type 1 diabetes in the general population. Genetic screening could be performed at birth to identify children at risk for type 1 diabetes, to develop a cost-effective and efficient strategy to determine who should be tested for predictive biomarkers (e.g., islet-specific antibodies) periodically throughout childhood. To aid in the identification of novel prevention strategies, it is also necessary to consider gene environment interactions such that preventative interventions may differ by genotype.

At present, genetic screening for risk assessment is used as a research tool to identify patients eligible to enroll in clinical trials. As prevention strategies are identified in the future, it will

be important to perform screening in the general population so that everyone can benefit from these new strategies. Increased knowledge about the genetic underpinnings of type 1 diabetes could enable the implementation of a public health program of immunogenic screening for pre-type 1 diabetes to eliminate the onset of mortality and morbidity and enable population-based primary prevention of type 1 diabetes.

- **Use genetic information to guide the selection of immunomodulatory treatment in new onset patients and islet transplant recipients.**

Based on the knowledge of disease-associated alleles in human and mouse type 1 diabetes, it is important to determine the function of these genes. This information could spur the development of assays that could be used to identify therapeutic agents directed at the gene product or other steps in pathways involving the gene product. These new therapeutic agents could be utilized in clinical trials that are based on the C-peptide reserve and immunosuppressive mechanism. Patients may participate based on a genetic risk profile.

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- **Test interventions aimed at the identified pathways in suitable animal models of type 1 diabetes and design human trials on the basis of this information.**

For disease-associated alleles in human and mouse type 1 diabetes, there is a need to discover the downstream consequences of gene variation through the application of molecular and cellular mechanistic studies. For human type 1 diabetes susceptibility genes and causative variants, there is a need to develop appropriate animal models with greater fidelity to human disease. The knowledge acquired relating the qualitative and quantitative effects of disease in animal models with the associated human variants will be critical for developing new targets for therapeutic interventions. Thus, advances towards realizing Goal I directly feed into the objectives and approaches described in Goal II.

Environmental Causes

During the 1950s and 1960s, the viral disease measles was widespread. In peak years during that era, 3-4 million cases of measles occurred in the U.S. population, resulting in more than 450 deaths annually (18). Vaccination programs have cut these rates dramatically. In recent years, fewer than 50 Americans developed measles per annum (18). The same strategy has led to the control of rubella, diptheria, tetanus, and mumps, the near elimination of polio, and the eradication of smallpox. Vaccines provide an excellent example of how prevention is more efficient and effective than treatment as a cure. What sets type 1 diabetes apart from the previously mentioned examples (all of which involve infectious disease with a known disease inciting agent) has been the lack of clear knowledge about which environmental agents promote type 1 diabetes; and, until recently, which beta cell autoantigen might be a reasonable target for such an effort. Identifying an environmental agent that promotes type 1 diabetes will depend to a large extent on the sucessful outcomes of another objective—to understand the interplay between early environmental encounters and the immunoregulatory defects, and how this interplay results in beta cell destruction in human type 1 diabetes (as discussed in Goal II). Specifically, with an improved identification of environmental encounters that modulate the processes of beta cell destruction in human type 1 diabetes, potential targets for vaccination should become more obvious.

Previous studies to investigate the environmental causes of type 1 diabetes have yielded inconsistent results, lagging behind studies of genetic causes of the disease, in part due to geographic differences, non-standardized measures, study design bias, and inadequate sample sizes. The TEDDY study will provide a coordinated, multi-disciplinary, and rigorous approach to this problem. Once environmental causes are definitively identified, strategies for the prevention of type 1 diabetes through clinical trials consortia, such as TrialNet (as described in Goal II), will be much more likely to be successful.

Pathogen detection techniques based upon high-throughput screening for presence of non-human nucleic acids and proteins have to be applied to serial samples obtained from children at risk for developing islet autoantibodies and clinical diabetes. It is plausible that one of the well-known and widely prevalent infectious agents (e.g., enterovirus) is responsible. If so, prevention of type 1 diabetes may require eradication of the agent in the population at-large. It is also possible that other viruses may be involved, or that viruses in general may trigger disease onset in genetically-susceptible individuals. Once environmental triggers are identified, significant effort will be required to develop vaccines or other prophylactic measures to lower the exposure and prevent disease onset.

Of critical importance is expertise in population-based infectious diseases epidemic modeling, including the role of rare viruses (or orphan viruses) on disease acquisition and transmission. Significant advances have recently been made in the area of pathogen detection. These technologies may offer unique opportunities in the search for unknown environmental factors that could trigger the autoimmune process in type 1 diabetes. In any case, understanding the triggering mechanisms, tissue tropism, and trafficking of cells will be important in providing insights into the mechanisms of disease initiation. Generating this knowledge will require attracting experts in these fields to pursue research on type 1 diabetes.

Long-term observational studies that will follow newborns in the general population with high-risk HLA genotypes, or newborns that have a first-degree relative with type 1 diabetes, are critical to understanding the importance of environmental factors triggering type 1 diabetes.

Research Objective—Monitor Rates of Type 1 Diabetes:

- **Monitor the incidence of type 1 diabetes in a representative sample of the U.S. population as well as from informative populations around the world to further define the course, and possibly the causes, of the recent rise in type 1 diabetes.**

Changes in the rate of disease in a population provide important clues about temporal environmental changes that may trigger development of disease. Although the U.S. has established the SEARCH consortium to assess the incidence and prevalence of all forms of childhood diabetes, the U.S. lacks immediate mechanisms to detect type 1 diabetes outbreaks and to monitor incidence rates--unlike other parts of the world that have established childhood diabetes registries.

Research Objective—Assess Environmental Causes of Type 1 Diabetes

- **Complete enrollment into the TEDDY Study, and begin well-powered nested case-control studies of TEDDY children who have developed persistent autoantibodies to GAD65, IA-2, or insulin to systematically evaluate candidate environmental causes of islet autoimmunity.**

The TEDDY consortium has developed a comprehensive multi-disciplinary and rigorous approach to this problem. Data are being gathered from cohorts of newborns identified to be at genetic risk for type 1 diabetes, both from the general population, and from first-degree relatives of patients with type 1 diabetes. These cohorts will be followed for 15 years for the appearance of various beta-cell autoantibodies and the development of diabetes. The TEDDY study will document maternal exposures, early childhood diet, reported and measured infections, vaccinations, and psychosocial stresses. Serial samples of serum, plasma, blood cells, mRNA, and stool samples will be taken from these children. TEDDY will also establish a central repository of data and biologic samples for subsequent hypothesis-based research.

- **Define further the effects of intrauterine environmental exposures (e.g., nutritional, infectious) on fetal and postnatal growth, islet development, and islet (beta cell) gene expression and function.**
- **Identify molecular genetic mechanisms by which specific environmental agents may trigger islet autoimmunity and promote progression to type 1 diabetes *in utero*, in early postnatal life, and later in development.**

The TEDDY protocol represents the current best effort at identifying testable hypotheses related to the etiology of type 1 diabetes. While identification of neonates at high genetic risk permits assessment of environmental exposures in infancy and childhood, intrauterine environmental factors may play a key role in setting the stage for diabetic autoimmunity. At present, very little is known about the immunology of pregnancy, or about the fetal and neonatal development of the immune system. To address this issue, the TEDDY Clinical Centers will screen and enroll pregnant women whose offspring are likely to be at increased risk because the mother herself, the father, or another child has diabetes. Blood samples from pregnant women will be obtained, and information on exposure to a potential trigger factor during pregnancy (e.g., an infection, preeclampsia, blood incompatibility, or birth weight) will be recorded to elucidate how intrauterine factors influence their children's risk of developing positive autoantibodies that are markers of type 1 diabetes.

- **Explore the possible role of emerging infectious agents, orphan viruses, and intestinal bacteria in the etiology of type 1 diabetes.**

The number of viruses infecting humans is increasing, and viral molecular genetics now permits detection of previously unrecognized infectious agents. However, at present, there is a poor understanding of the mechanisms by which micro-organisms colonize the human gut and influence the gut-associated lymphoid system. More effort is needed at a basic level to understand mucosal immunity relevant to autoimmunity, as discussed in Goal II. Further studies are warranted on the close association between type 1 diabetes and celiac disease, as well as the relationship between early exposure to gluten and appearance of islet autoantibodies.

- **Translate novel findings about reduced herd immunity through specific vaccination in the general population and relate this to a possible decrease in herd immunity to common viruses such as human enteroviruses.**

Judicious childhood vaccination practices have not reduced the incidence rate of type 1 diabetes in children. At the same time, human enterovirus infections that were formerly pandemic are no longer affecting a large proportion of the population. Reduced exposure is reducing herd immunity. It has been proposed that type 1 diabetes mimics the polio virus epidemic in which a reduced exposure lowered maternal immunological protection leading to poliomyelitis in less immunologically protected children.

- **Explore candidate environmental agents (e.g., food elements, toxins, and infectious agents) as triggers for islet autoimmunity and type 1 diabetes in animal models of type 1 diabetes.**

Knowledge about the effects of dietary manipulation, metabolic stress, or viral infection on the development of spontaneous type 1 diabetes in rodent models will be critical for elucidating the molecular mechanisms of environmental triggers, and for developing new therapies to prevent the disease. Thus, a greater understanding of type 1 diabetes etiological mechanisms included in this Goal is entwined with the Goal II objective to better understand the regulation of immune responses in type 1 diabetes. Good animal models are a critical means to both ends.

- **Establish a resource of biological materials that will facilitate research on the environmental basis of type 1 diabetes in the older population.**

Although long-term observational studies are being carried out in younger populations, little information exists regarding the environmental causes of type 1 diabetes in those who develop the disease later in life. Clearly, such studies will require longer term commitments and broader screening protocols than have yet been employed.

Table 1. Key Research Objectives for Identifying Genetic and Environmental Causes of Type 1 Diabetes

- Create Resources for the Study of Type 1 Diabetes Genetics
- Identify Human Genes Causing Type 1 Diabetes Using the Candidate Gene Approach
- Use Knowledge About the Genetic Underpinnings of Type 1 Diabetes to Prevent and Treat the Disease
- Monitor Rates of Type 1 Diabetes
- Assess Environmental Causes of Type 1 Diabetes

GOAL II: PREVENT OR REVERSE TYPE 1 DIABETES

Why This Goal Is Important to People

PARENTS OF CHILDREN AT RISK FOR DEVELOPING TYPE 1 DIABETES ASK: "CAN RESEARCH HELP MY CHILD LEAD A LIFE FREE OF THIS DISEASE?" THOSE WHO ARE NEWLY DIAGNOSED ASK: "IF MY BODY IS STILL MAKING INSULIN, WHAT CAN I DO TO PROLONG THIS?"

Insulin treatment is essential for the survival of type 1 diabetes patients, but it is not a cure. For the rest of their lives, patients must carefully watch their food intake, monitor their blood glucose levels, and try to control them with externally administered insulin. More serious than the inconvenience and discomfort of this treatment regimen are the danger of acute life-threatening episodes of low blood glucose, and the very high probability of chronic, disabling complications. To end these problems, researchers seek to short-circuit the underlying autoimmune disease process—that is, to thwart the immune system's misguided destruction of insulin-producing pancreatic cells.

Because the genetic and environmental causes of type 1 diabetes are not well understood, strategies to prevent or reverse the disease are currently focused on intervening in the immune system's assault. These strategies must be two-pronged; they must squelch autoimmunity in those who are at risk for or already have the disease, while maintaining or restoring the patient's own insulin-producing capacity. (Goal III addresses another approach for reversing the disease by transplanting insulin-producing cells obtained from donor pancreatic tissue or regeneration of beta cells.)

Of course, the immune system provides critical protection against infection, so it is vital for any approach that modifies its activities to be as selective as possible in damping down just those processes that lead to autoimmunity. This delicate balancing act will be achieved by leveraging knowledge about the immune system in general, combined with insights into disease causation, in order to devise new diagnostic, treatment and prevention strategies. Success will depend in large measure on building upon research advances and pursuing opportunities for uncovering the roots of this disease. Fortunately, research on type 1 diabetes has already advanced to the point that some new prevention and reversal strategies can be tested even in the absence of complete knowledge of disease causation.

Major progress has also been achieved through the identification of antibodies that are produced in type 1 diabetes when the immune system attacks the body's insulin-producing cells. These antibodies are therefore markers of type 1 diabetes. Importantly, they have been shown to be detectable well before the loss of insulin-production and the diagnosis of clinically overt disease. Tests of these antibodies in the siblings of type 1 diabetes patients can predict with great reliability whether they too will develop the disease in a few years. This predictive tool, coupled with other new technologies, has given researchers the remarkable ability to design and conduct primary prevention clinical trials in type 1 diabetes. A major research goal is to expand this type

of predictive tool beyond its current, limited use in first degree relatives of type 1 diabetes patients.

Understanding Regulation of the Immune System

The immune system is made up of a complex army of different cell types that, when functioning properly, interact with one another to respond to various threats. Certain of these cells act to regulate the function of others. A central feature of that regulation is a process called "tolerance"—recognition of self—which prevents the immune system from attacking the body's own cells. Tolerance is therefore a major focus of research on all autoimmune diseases, and scientists are making important strides in understanding how it works. Studies in special mouse and rat strains that have a genetic predisposition to type 1 diabetes have been insightful and will continue to contribute significantly to this research. While many forms of white blood cells play important roles in the autoimmunity of type 1 diabetes, researchers are homing in on the functions of one cell type (the T cell), which is thought to be instrumental in the autoimmune process due to its destructive capacity combined with its potential to affect immune responses. For years, researchers have struggled to determine which beta cell proteins are key targets of autoimmune attack. Several published studies now support the notion that the insulin molecule itself is an important, potentially type 1 diabetes-initiating target of the immune system, although several other proteins may also play a role.

Preventing Type 1 Diabetes and Prolonging Pancreatic Function

A more thorough understanding of genetic factors underlying type 1 diabetes could lead to preventive approaches. Similarly, knowledge of disease-triggering environmental factors would help researchers find ways to eliminate or compensate for exposure to them—for example, development of a vaccine if viruses are implicated. At present, however, knowledge of causative factors is incomplete, and therefore, strategies to prevent or reverse the disease are directed largely toward modulating the autoimmune process. Research has progressed to the point where it is increasingly possible to identify some type 1 diabetes patients in the earliest stages of the disease, when they still have a significant fraction of their insulin-producing cells alive and functioning. A treatment that prolongs pancreatic functioning during this period would be a great boon to patients, because they could achieve better blood glucose control with less risk of hypoglycemia. Researchers are now testing several promising treatment regimens based on this approach.

Improving Screening for Type 1 Diabetes Risk

Prolonging pancreatic function can only be an effective strategy if health care providers are able to identify patients in the early stages of autoimmunity. Researchers are making progress in identifying genes associated with type 1 diabetes, many of which may play a pivotal role in regulating the immune system. Acquiring full knowledge of the genes that protect against or dispose to the disease will enable researchers to develop interventions targeted to those genetic pathways. Likewise, improvements are being realized in screening methods to detect those in whom autoimmunity is at its earliest stages. Current screening strategies look for proteins, called autoantibodies, which characterize the autoimmune attack. Tests of T cell function are under

development as useful tools for monitoring autoimmunity. New imaging methods are also in development to detail the immune process in the pancreas. These methods may not only be excellent screening tools, but may also help scientists better understand the biology of autoimmunity.

Reversing Type 1 Diabetes

Suppressing autoimmunity must be a key component of any treatment designed to reverse type 1 diabetes in affected individuals. Once this suppression is accomplished, it may in principle be possible for patients to re-grow pancreatic tissue. Indeed, data from the landmark Diabetes Control and Complications Trial suggest that some people with type 1 diabetes maintain a limited capacity to produce their own insulin long after onset of disease. This finding is confirmed by recent research showing that most people with type 1 diabetes, regardless of length of disease, still have beta cells. These findings could mean that some beta cells are escaping the immune attack, and that their activity could possibly be bolstered. Alternatively, and perhaps more likely, the pancreas may be able to sometimes counter autoimmunity through a limited capacity for regeneration—although not nearly to a sufficient degree to actually offset beta cell losses. Unfortunately, many drugs that are known to be effective in suppressing the immune system (most commonly used in transplant patients) are actually harmful to beta cells, and may prevent regeneration. Researchers are therefore attempting to develop new medications that are gentler, and better targeted to the immune cells most directly responsible for beta cell autoimmunity. Medical approaches to stimulating beta cell development and function are addressed in Goal III.

Identifying and Optimizing Detection of Immunologic, Genetic and Metabolic Disease Markers

With precise genetic markers, screening methods that are currently applied only to relatives of type 1 diabetes patients could conceivably be enhanced to predict and monitor disease risk in the entire pediatric population or the general population. Such genetic risk assessment would also shed light on other autoimmune diseases that often occur in type 1 diabetes patients, including celiac disease, Addison's disease, and rheumatoid arthritis.

Determining How Destruction of Insulin-Producing Cells Results from the Interplay Between Early Environmental Encounters and Defects in Regulation of the Immune System

Innovative clinical research studies will be made possible through a better understanding of facets of the immune response (e.g., regulatory T cells, innate immunity, etc.), which have recently been appreciated as key mediators of beta cell destruction. For example, it is important to understand cells that regulate the immune response; to develop better assays to measure the autoimmune response; and to find ways to measure the mass and function of insulin-producing cells.

Devising Targeted Interventions Capable of Long-Term Reversal of Recent-Onset Type 1 Diabetes without Concomitant Short- or Long-Term Adverse Effects

Research has already demonstrated that many interventions can prevent type 1 diabetes in the animal models that spontaneously develop the disease, but fewer have shown promise of reversing the disease. Unfortunately, clinical testing of agents has been limited, and no intervention found to be effective in animal studies has yet proven effective in humans. Progress would be furthered by the conduct of multiple clinical trials—based on sound infrastructural support and incorporating standardized design and outcome measures. Trials would assess not only drug efficacy, but also safety considerations—particularly the safety of agents that modulate or suppress the immune system. It would also be beneficial to determine whether combination therapies offer improvements over therapies directed solely at the immune system. Additional studies should evaluate whether the preservation of insulin-production in recently diagnosed patients offers short and long-term clinical benefit with respect to complications—particularly eye, kidney, and nerve disease.

Enhancing Animal Models

Additional animal models are needed to accelerate the study of relevant immune mechanisms and potential interventions. While spontaneous animal models of type 1 diabetes have been very useful in understanding the mechanisms underlying development of the disease, some strategies that prevent it in animals have not proven successful in humans. Thus, mouse models that mirror the disease in humans with greater fidelity than do current models should be derived and tested for their utility to serve as surrogates for investigating new therapies aimed at combating autoimmunity. Such work would enhance the development of safe compounds for later testing in type 1 diabetes patients.

Developing a Safe and Universal Means for Primary Prevention

Vaccination programs have dramatically cut rates of infectious diseases such as measles, rubella, diphtheria, tetanus, mumps and polio. These examples clearly show how prevention is more efficient and effective than treatment. With an improved identification of environmental factors that modulate autoimmunity, potential targets for vaccination should be revealed for prevention of type 1 diabetes in humans.

GOAL II: PREVENT OR REVERSE TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

In type 1 diabetes, the immune system attacks and destroys the insulin-producing beta cells within the pancreas—treating them as if they were infectious invaders or an organ transplanted from another individual. The immune system is normally well regulated against the formation of self-directed or "autoimmune" processes due to the body's remarkable ability to form "tolerance," a process whereby cells of the immune system are either eliminated or turned off if they react to one's own cells or proteins. Yet, for unknown reasons, this process of immunological tolerance fails to work properly in persons who develop type 1 diabetes, thereby permitting the self-destruction of beta cells. As discussed in Goal I, research suggests that this autoimmune attack may be triggered and/or exacerbated by as yet unknown environmental factors in persons who are genetically at increased risk for developing the disease, but the specific roles of genetics and environment in the pathogenesis of type 1 diabetes remain unclear.

An individual's level of genetically-encoded risk for developing type 1 diabetes aside, the earliest marker that portends ultimate beta cell destruction is the appearance in the bloodstream of antibodies (i.e., autoantibodies) that recognize "self" beta cell proteins. In type 1 diabetes, autoantibodies are not themselves thought causative of disease--as they are in myasthenia gravis, for example. Instead, they are thought to result indirectly from the cell-mediated immune destruction of the pancreas, often referred to as the white blood cell response. This is not to say that autoantibodies are without clinical or diagnostic value in type 1 diabetes. Indeed, they have been used as highly effective biomarkers for identifying individuals who are in pre-clinical stages of the disease, and have served in the biochemical definition of the self-proteins that are targets of immunological attack. While many forms of white blood cells play important roles in the autoimmune processes that damage beta cells (e.g., macrophages, dendritic cells, etc.), a key role has been suggested for T cells (also called T-lymphocytes)—a cell type that, in addition to having destructive capacity, also has the potential to limit immune responses.

Type 1 diabetes research is fortunate to have not just one but several spontaneous rodent models of the disease. These animals serve as excellent surrogates for evaluating the mechanisms underlying type 1 diabetes, as well as for testing agents capable of reversing the autoimmune processes mediating beta cell destruction. The BB rat and the NOD mouse are the most prevalent models, at least as evidenced by the number of publications emanating from their use. Both animal models share many characteristics with human type 1 diabetes, including: genetic susceptibility by molecules regulating the immune response; white blood cell infiltration of the pancreatic islet cells; a disease that is influenced by environmental encounters; and the production of autoantibodies against beta cell proteins. Furthermore, in both models, beta cell destruction can be attenuated through application of agents capable of influencing the immune response.

Based on the present state of knowledge, a cure for type 1 diabetes will hinge on the ability to interrupt the destructive assault by the cell-mediated immune system. Such interruption will be necessary whether the goal is to stop the disease before it progresses to full-scale loss of

pancreatic endocrine function (i.e., avoiding symptomatic onset and need for insulin therapy), to reverse type 1 diabetes, or to prevent the recurring immune attack on islet beta cells following their transplantation into patients with long-standing type 1 diabetes. Indeed, a central problem that must be solved is the development of a method that promotes the induction of immunological tolerance to pancreatic beta cells in persons genetically predisposed to type 1 diabetes. It should also be emphasized that basic as well as applied research will be of critical importance for achieving this goal.

RECENT SCIENTIFIC ADVANCES

While attempts to turn advances in understanding the autoimmune basis for type 1 diabetes into a cure have been made for nearly three decades, more progress has been achieved in the last five years than in the previous 25 years combined. Only recently have researchers realized that the autoimmune processes associated with type 1 diabetes begin for many in the first months to years of life (i.e., when the aforementioned autoantibodies form). Likewise recent is an improved appreciation of the ability of environmental factors (e.g., diet, viruses) to influence, in a positive or negative way, the rate of progression to type 1 diabetes. Through studies of both humans and of animal models of type 1 diabetes (e.g., BB rats, NOD mice), dramatic improvements have recently occurred in understanding the "basic" immunologic mechanisms that, acting in concert, contribute to the dysregulated immune response that results in loss of tolerance, beta cell destruction, and eventually, type 1 diabetes.

Tolerance and Regulation of the Immune System: Recent studies of animal models have provided insights into type 1 diabetes such as:

- Ascertaining the physiological locations of the defects that underlie the failure to develop tolerance to beta cells (i.e., the role of the thymus gland *versus* cells of the immune system that circulate through the peripheral immune system);
- Identifying immune system cells that are key to inducing tolerance in type 1 diabetes (e.g., B-lymphocytes, dendritic cells, regulatory T cells); and
- Pinpointing the contributions that various cytokines (i.e., chemical signals of the immune response) make to the onset and progression of this disease.

It is important to note that currently, many of these disease aspects can only be addressed through studies of animal models due to issues of both practicality and technical ability; providing but one of many examples of the importance of animals to type 1 diabetes research. Progress toward understanding tolerance and regulation of the immune response in human type 1 diabetes has also occurred, implicating defects in many cell types (e.g., regulatory T cells, dendritic cells, natural killer T (NKT) cells) as potentially causative in autoimmune disorders such as type 1 diabetes. Similarly, several genes (e.g., AIRE, and others derived from the genomic analyses described in Goal I) have been identified that contribute to autoimmune disorders because of their ability to modify immune reactivity.

Identification of Autoantigens and Improved Tools for the Study of Type 1 Diabetes Onset: For years, researchers have struggled to determine which beta cell proteins are key targets of autoimmune attack. A variety of investigations, in both animal models and humans with type 1

diabetes, now support the notion that the insulin molecule itself is an important, potentially disease-initiating autoantigen in this disease. Additionally, other studies have recently identified islet specific glucose-6-phosphatase catalytic subunit related protein (IGRP) and dystrophia myotonica kinase (DMK) as antigenic targets of the cellular immune response in NOD mice. There is also continuing interest in the potential role that proteins of neuroendocrine origin may play in the disease (e.g., glutamic acid decarboxylase, IA-2, phogren) in both human type 1 diabetes, as well as in animal models. To a large extent, many of these recent discoveries regarding autoantigen identification were dependent on the development of improved tools for characterizing the immune response associated with beta cell destruction (e.g., T cell tetramer and ELISPOT assays, genetically modified mouse models of type 1 diabetes, etc.), as well as on access to human tissues made available for research purposes (e.g., islet cells, pancreas, and pancreatic lymph nodes from type 1 diabetes patients). In addition to immune markers, a variety of metabolic markers and their associated tests have also proven valuable to studies of human type 1 diabetes. Particularly notable are the recent improvements in the ability of researchers to determine the metabolic activity of individuals with or at-risk for type 1 diabetes (e.g., C-peptide standardization).

Advances in Preventing or Reversing Type 1 Diabetes: Recent years have seen much excitement about possible treatment strategies stemming from proof-of-principle experiments in animal models. These include, but are not limited to: anti-CD3, which depletes and/or modifies the function of T cells; anti-CD40L, which antagonizes immune activation, e.g., "costimulatory blockade;" and anti-thymocyte globulin, which also depletes T cells. In addition, research on immunosuppression associated with the islet transplantation efforts, as described in Goal III, also contributes leads for agents that could be used to control autoimmunity in the disease prevention or reversal setting. Those agents that demonstrate adequate safety profiles have and will continue to move forward in human type 1 diabetes clinical trials, through such programs as NIH's Type 1 Diabetes TrialNet or the Immune Tolerance Network (ITN). Anti-CD3 is one example of an agent that has seen experimental translation from animal models to investigations in humans. Two research trials of anti-CD3 reported the ability of this agent to preserve metabolic function when administered to people with recent onset type 1 diabetes. With time, it is hoped that this or other agents will become proven components of a cure for type 1 diabetes by promoting disease reversal.

Studies of animal models of the disease, as well as investigations of its natural history in humans, have generated a number of agents or practices that could be useful for preventing the disease in those with a high-likelihood of developing it (e.g., omega-3 fatty acids, cow milk avoidance, etc.). In some situations, methods used for disease prevention may be similar to or the same as those for type 1 diabetes reversal. However, it also appears that a "one size fits all" approach to type 1 diabetes therapy will not be practical. Studies of animal models suggest that optimizing therapeutic efficacy may depend on tailoring the therapy for each point in the disease process.

In terms of attempts to prevent the disease, a degree of disappointment obviously surrounds the results of the DPT-1 clinical trial, which was conducted in relatives of type 1 diabetes patients who do not themselves have the disease but who have signs of autoimmunity. The trial found that insulin administered via daily injection did not prevent type 1 diabetes in those at increased risk for the disease. However, a number of positive research outcomes were and continue to be

seen from that effort. First, the trial instilled an appreciation that very meaningful scientific information can be gleaned from trials even when prevention of disease may not occur. For example, there was an observable nationwide confirmation of the practical ability to use autoantibody and genetic markers of type 1 diabetes to predict future cases of the disease. Because physicians can effectively identify those individuals at increased risk for the disease, they are in a better position to fight the disease when superior interventions are developed. In addition, although injected insulin was ineffective, the trial suggested that oral insulin administration may have a potential benefit with respect to delaying disease in a select group of those identified as being at intermediate risk. This approach will be tested in a future effort using the TrialNet consortium.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

Finding a means to prevent or cure type 1 diabetes will require an accurate assessment of what is *truly* known about the disease in humans, as well as an organized plan to fill the knowledge voids that stand between the diabetes community and that goal. To that end, the following objectives are critical.

Risk Assessment

The DPT-1 Trial affirmed, at a national level, the ability to identify individuals at increased risk for future development of type 1 diabetes. This study--in a patient population of relatives (non-diabetic but having signs of autoimmunity) of type 1 diabetes patients--was built on years of experience in smaller trials indicating the value of screening for type 1 diabetes using combinations of autoantibody, genetic, and metabolic markers for the disease. Despite this success, the prediction of type 1 diabetes largely remains limited both in scope of application (i.e., who is screened) and in the locations in which such testing occurs (i.e., within academic research settings). Furthermore, practical improvements in the technology of disease prediction would be of immense benefit, as would better integration of additional physiological parameters (e.g., body mass index (BMI), age, etc.) to enhance existing predictive models.

Indeed, a large majority of studies to date have focused on screening relatives of individuals with type 1 diabetes. This focus is understandable in terms of efficiency (the risk of type 1 diabetes in close relatives of those with the disease is approximately 1 in 25, while in the general population it is around 1 in 300 (14). However, more than 80 percent of new-onset type 1 diabetes patients do not have a known family history of the disease (14). Also, it remains to be seen whether the disease characteristics of patients from the general population differ from those identified in family groups—differences that could have impacts on the efficacy of a proposed treatment or prevention. Hence, it would be wise to initiate studies testing the feasibility of population-based screening in order to identify at-risk individuals from the pediatric population as a whole. Also, while type 1 diabetes screening is efficacious, for the most part it remains a research-based effort performed in a limited number of academic research centers. While such institutions certainly play a key role in type 1 diabetes care, only a small percentage of the people receive health care in such facilities. Therefore, it would be valuable to develop point-of-service screening for type 1 diabetes risk, such that these assays could be performed in pediatricians' offices (e.g., using a finger-stick blood test). It must be emphasized that this forward-thinking objective remains

research-based and must, if implemented, be undertaken with training and education for health care providers, as well as the general public. Such efforts must also be accompanied by the full intent to investigate issues of patient privacy, family counseling, and ethics.

Technological improvements must go hand-in-hand with broad access to samples to promote the development and validation of risk assessment technologies. Past experiences suggest that assembling and tracking large collections of samples require considerable investment and buy-in from study investigators from the very beginning, and careful consideration of issues related to patients' informed consent and privacy. Large multi-center clinical studies and trials (such as TrialNet and TEDDY) are already collecting and archiving sample banks to be made available to the research community. An important priority is to continue and to expand these efforts to promote access and efficient distribution of samples to the research community.

Research Objective—Identify and Optimize the Detection of Immunologic, Genetic and Metabolic Markers of Type 1 Diabetes:

- **Improve measurement of autoantibodies and other autoimmune markers to identify those at risk in the general population.**

Intensive efforts should be directed to miniaturizing existing technologies for assessment of immune activities related to type 1 diabetes development. Specifically, diagnostic tests should be developed that require smaller blood volumes than are currently necessary, and permit collection under conditions that do not require vein puncture (e.g., capillary tube collection or spotting of blood samples on filter paper). Such improvements would facilitate more frequent monitoring of patients, leading to discoveries of changes in the immune response that are not currently observed with existing collection schedules (e.g., quarterly or semi-annually). Improvements in technologies of a different sort could also enable much needed improvements in assays for anti-insulin autoantibodies, as well as the identification of any additional previously unknown beta cell autoantigens. As previously indicated, while autoantibodies represent important and proven markers of type 1 diabetes, the processes underlying the disease likely reside in components of the cellular immune response. Earlier attempts to use cellular immune markers for type 1 diabetes screening have, in a majority of situations, proven difficult in terms of technical reproducibility and practical issues. This situation must change. Fortunately, new technologies are being developed that could provide the needed, more powerful biomarkers. These technologies include genomics (examples provided in Goals I and VI), proteomics (discussed in Goal VI), RNA markers, and the quantitative measurement of cytokines in blood.

- **Refine type 1 diabetes risk assessments by exploiting additional genetic markers.**

Given that new technologies will also continue to revolutionize genetics, future studies should determine whether additional genetic markers could refine and improve existing algorithms for type 1 diabetes prediction. Many of the opportunities and challenges within this area of research were described under Goal I. Genetic risk assessment for type 1 diabetes should also be expanded to define the risk for a series of other autoimmune disorders (e.g., celiac disease, Addison's disease, rheumatoid arthritis, etc.) that often occur in patients with type 1 diabetes, with the potential benefit of affording primary, secondary, or early tertiary intervention for these related disorders to reduce their disease-associated morbidity and mortality.

- **Refine type 1 diabetes risk assessment using metabolic parameters.**

In addition to improvements in immunologic and genetic markers, similar efforts for discovery should be aimed at enhanced understanding of metabolism in the type 1 diabetic setting, in the period prior to symptomatic onset, as well as at disease diagnosis. Specifically, studies should examine a variety of physiologic variables (i.e., age, BMI, insulin resistance, etc.) with the aims of improving understanding of their contribution to the heterogeneity of this disease, and designing targeted therapies that might prove more effective given a specific set of immunologic and physiologic criteria. Additional efforts should also be directed at continuing the process of standardizing C-peptide response to metabolic stimulation as a measure of beta cell function and addressing outstanding questions such as: what should be measured; which test should be used to measure it; and when should the test be administered?

Immunopathogenesis

While studies on the natural history of type 1 diabetes have not yet resulted in a means to prevent or cure the disease, they have led to a remarkable improvement in understanding the events prior to the symptomatic onset of disease. As previously mentioned, persons identified to be either at low or high risk in DPT-1 were characterized as extensively as possible (within the logistical, ethical, and scientific constraints) to identify molecular and cellular markers that indicated a high likelihood of progression to overt type 1 diabetes. While the ability exists to stratify individuals at birth for their risk for type 1 diabetes, studies of the natural history of type 1 diabetes in early childhood and adolescence (such as the TEDDY study and the Natural History study within TrialNet) clearly need to continue. Such studies address the need to know more about the role of the environment in the pathogenesis of the disease, as well as to provide a more detailed characterization of the immune system abnormalities that result in beta cell destruction.

Research Objective—Understand the Interplay Between Early Environmental Encounters and the Immunoregulatory Defects Which Results in Beta Cell Destruction in Human Type 1 Diabetes:

- **Improve understanding of the interplay between the environment and the immune system, which leads to the autoimmune destruction of beta cell in humans.**

For decades, investigators have sought to identify "the type 1 diabetes virus." However, recent research suggests that there is a complex liaison between viral infections and other potential environmental triggers of type 1 diabetes, as discussed in Goal I. Only recently have researchers begun to appreciate the possibility, based on animal models, that some environmental agents may not enhance disease progression, but rather, may offer protection from disease. Hence, it has now become of paramount importance to define experimentally the scenarios that can potentiate acceleration of beta cell destruction *versus* those that can dampen autoimmune beta cell destruction. Because these are exceedingly difficult studies to perform, close collaboration among a number of large, prospective research efforts is necessary, such as the coordination provided by the NIH TEDDY consortium (described in Goal I). These collaborative efforts will promote efficient investigation of important issues, such as assessing in infants the timing of cereal introduction as an influence in the development of anti-islet autoimmunity. It would be beneficial to capture individuals

undergoing anti-beta cell immunity at the height of an inflammatory event in epidemiological studies and not only at set-time intervals. Additional reasons to continue studies on the natural history of type 1 diabetes include the need to establish whether type 1 diabetes is, like other immune mediated diseases, a disease of flares and remissions. Finally, researchers need to gain a better understanding of the interaction of the innate and adaptive immune system in early human development, as well as the role of gut immunity in the development of type 1 diabetes.

- **Create a database of the genes expressed in the pancreas at sequential stages of type 1 diabetes development, as well as accessible tissues involved in the (auto)immune response.**

Substantial research into gene expression and proteomics will be required to translate findings from T1DGC and TEDDY into new molecular diagnostic tests to help physicians predict type 1 diabetes, determine the stage of islet autoimmunity, select preventive measures, and monitor therapies. Some of the genetic markers will be considered as potential therapeutic targets for new drugs. Microarray experiments are providing unprecedented quantities of genome-wide data on gene-expression patterns, but the management and analysis of the millions of data points that result from these experiments will require sophisticated new computational tools. These tools should be utilized in studies to: assess levels and patterns of gene expression in each tissue before and after appearance of islet autoantibodies and autoreactive T-cells and before/after candidate environmental exposures; correlate the level and patterns of expression at the mRNA and/or protein level with the genetic and metabolic phenotypes of humans and animal models before and after disease onset; and generate expression analyses from a panel of humans and laboratory animals at different stages of type 1 diabetes. The latter effort should focus on the genes most likely involved in environmental triggering of islet autoimmunity and progression to overt diabetes to determine the range of sequence and expression variation in these genes and the proteins they encode.

Research Objective—Advance Basic Understanding of Facets of the Immune Response (e.g., regulatory T cells, innate immunity, etc.) that Have Recently Been Appreciated as Key Mediators of Beta Cell Destruction:

- **Improve the understanding of the generation and function of regulatory T cells in type 1 diabetes.**

In the mid-1970s and early 1980s, studies of animal models of type 1 diabetes suggested a key role for T cells in the processes of beta cell destruction. For nearly two decades, the mechanisms by which these cells could act in both a destructive and protective fashion remained enigmatic. However, within the last five years, research has highlighted the role of a population of T cells commonly referred to as "regulatory T cells," a form of the white blood cell that may represent one of the master regulators of the immune response. Studies in NOD mice, BB rats, and human type 1 diabetes patients suggest important pathogenic and therapeutic relationships between these regulatory T cells and disease. It is imperative to determine the role of regulatory T cells in the natural history of type 1 disease. Lack of understanding about the cellular immune response in general (and T cells in particular) represents one of the most serious gaps in knowledge that must be filled to realize the goal of prevention and reversal of type 1 diabetes.

- **Develop better assays to measure the autoimmune response and to serve as biomarkers of response to therapy.**

One possible approach to this objective would be to develop assays with animal models, using blood taken from a human patient, to detect and quantify T-lymphocytes capable of inducing type 1 diabetes. Similar assays (using animal models – both rodents and non-human primates) could be developed to monitor immunologic therapies for type 1 diabetes. These assays would provide benefits by both identifying the most efficacious agents and by predicting response to therapy. Improved cellular immune assays are also needed to determine the metabolic and immunologic events that occur during transition from pre-symptomatic to overt disease. Likewise, these assays will be important in determining the relationship between genotype and phenotype in humans, particularly with respect to immunologic function. It should be emphasized that the need for improved assays for these purposes is especially required for monitoring cell-mediated immunity in peripheral blood from patients enrolled in clinical trials. Development of assays of immune activation and/or tolerance is a key objective described in Goal III. It is likely that common approaches can and will be used to study both autoimmunity and alloimmunity relevant to transplantation.

- **Detect and measure the autoimmune response, and the mass and function of beta cells, at the level of the pancreatic islet.**

While diagnostic or research-oriented sampling can safely be accomplished in certain cases, for example, rheumatology or kidney transplantation patients, pancreatic biopsy is neither safe nor practical in individuals with or at risk for type 1 diabetes. However, it is critically important to identify both the destructive T cells, as well as the molecules that they recognize, that infiltrate islets and pancreatic lymph nodes of humans with type 1 diabetes or those in the process of disease development. Recently, an initiative has put in place an international network of centers with the ability to screen deceased individuals for detectable islet autoantibodies, and obtain from those positive individuals pancreatic and nearby immunologic tissue. This effort may seem like a “needle in the haystack” problem, but represents the only and best means for obtaining this essential material resource. Other efforts have been directed at improving the ability to image *in vivo*, non-invasively and safely, but with high resolution, the degree of beta cell mass and the quantity of islet infiltration and inflammation. Aside from further efforts to understand damage inflicted on beta cells by the immune system, additional studies should be directed toward examining the effect of hyperglycemia (independent of immune attack) on beta cell destruction and growth. Non-invasive imaging, both for islet cell mass and function, as well as inflammation or immune infiltration, is a goal common to diabetes prevention/reversal and islet transplantation efforts.

Clinical Trials

Interestingly, a great many interventions have been shown capable of preventing type 1 diabetes in the animal models that spontaneously develop the disease. Fewer have been shown capable of reversing type 1 diabetes in animals, and fewer still have been tested for their capacity to prevent or reverse the disease in humans. Selected examples range from those with a dietary/environmental basis (e.g., nicotinamide, delayed introduction of cow’s milk) and

immunosuppression/immunoregulation (e.g., cyclosporine, anti-CD3) to those that have an antigen specific immunomodulatory function (e.g., oral and subcutaneous insulin).

Considerable evidence suggests that administration of a variety of beta cell autoantigens can delay the onset of type 1 diabetes in animal models of the disease. For example, some studies point to insulin as a beta cell autoantigen with potential pathogenic significance. While the DPT-1 study did not support the ability of injected insulin to prevent type 1 diabetes, a number of distinctions exist between the tested therapy and the use of a putative insulin vaccine. Among them would be aspects related to form (e.g., insulin peptides, the use of adjuvants to stimulate immune responses, route of delivery), function (i.e., type of immune response one wishes to elicit), and time of administration (i.e., early in life *versus* the late administration employed in the DPT-1 trial). To be clear, studies of autoantigen administration should not be limited to insulin. Moreover, the impact of such trials may extend beyond that of universal/early administration and extend to therapies of recent-onset type 1 diabetes patients.

The current state of knowledge offers several agents with therapeutic potential, but no single agent is clearly most worthy of testing for the prevention of type 1 diabetes. Thus, achieving this objective will involve multiple clinical trials. Such trials should not only test efficacy in terms of type 1 diabetes prevention or reversal, but also assess the ever important safety considerations. Efforts are currently under way to implement well-organized clinical trials, and to establish and maintain an efficient infrastructure for the identification of populations for participation in research. Moreover, the next phase of type 1 diabetes prevention trials will benefit from lessons learned through previous attempts to achieve type 1 diabetes prevention or reversal.

Knowledge gains stemming from the NIH funded Diabetes Control and Complications Trial about the health benefits of even low levels of residual beta cell function are furthering efforts to achieve type 1 diabetes prevention or reversal. Extremely beneficial would be the identification of an intervention, or combination of treatments, capable of either inducing complete disease remission or perhaps even prolonging the "honeymoon" phase during which new-onset patients still have meaningful beta cell function. Such an interventional strategy could not only have a dramatic impact on a patient's daily lifestyle, but might also delay or prevent the development of complications associated with the disease. Indeed, the development of a method for type 1 diabetes reversal would have an immense impact on the over 13,000 young Americans that are newly diagnosed with type 1 diabetes each year (11).

Research Objective—Identify an Intervention Capable of Long-term Reversal of Recent Onset Type 1 Diabetes Without Concomitant Short- or Long-term Adverse Effects:

- **Standardize trial design and outcome measures.**

Information gathered in clinical trials will be most useful if a standardized approach to data collection is taken and adhered to across the participating clinical centers and even across clinical trial and study consortia. This standardization will require cooperation and communication among researchers at every level. Standardization of measures employed in the trials must be undertaken and implemented in an ongoing way. These include measurements of autoantibodies, including titers and affinities; cellular-based measures of autoimmunity; measures of inflammation; and metabolic measurements, including C-peptide and hormone production, insulin usage, and glycemic control. Trial design considerations,

such as issues of "effect size" and power calculations, will also need to be examined and implemented consistently across trial consortia.

- **Determine whether combination therapies offer improvements in terms of efficacy over monotherapies directed solely at the immune system.**

As already proven in oncology, combination treatment methods may limit adverse side effects while improving efficacy. One particularly promising combination therapy approach to type 1 diabetes would be to test immunomodulating agents along with potential beta cell "growth factors" (e.g., an incretin mimetic or growth hormone). Another example would combine a tolerance induction methodology with an immunosuppressive approach to reverse anti-beta cell autoimmunity. Emphasis should also be given to studies that combine immune intervention agents with drugs that send survival signals to islet beta cells (inhibiting programmed cell death), leading to a preservation of existing beta cell mass and improved beta cell growth. Antigen-specific interventions should also be combined with non-specific immunosuppressants. The former have the advantage of site-specific and non-systemic action, while the latter offer an immediate attenuation of anti-beta cell immunity. Such combination therapies would also be clearly relevant to the field of islet transplantation as described in Goal III.

- **Identify novel therapeutic agents.**

While it is true that many potential therapies for type 1 diabetes reversal exist, there remains a pressing need for additional candidates, including those that could promote a "costimulatory blockade" or an induction of regulatory T cells. For any effort in this area to succeed, it is important to identify groups (academic, corporate, other) that are highly proficient and competent in rational drug design and willing to work with the type 1 diabetes research community to either create novel immune interventions or find new applications for existing drugs. Such efforts will help overcome existing barriers that inhibit large pharmaceutical companies focused on larger markets from committing to high-risk projects such as some of those described in this Strategic Plan.

- **Assess the safety of all immunomodulating or immunosuppressive therapies tested in type 1 diabetes.**

Recent research on immunosuppression in autoimmune diseases has revealed not only impressive potential benefits, but also great potential risks. Clearly, the risk/benefit equation in a prevention setting is very different from that in a life-saving organ transplant situation. A major research aim is to analyze the effects of immunosuppression on immunization status, viral activation or reactivation. For example, one of the most feared complications in a chronic disease such as type 1 diabetes is reactivation of viruses that could have long-term oncogenic potential. Indeed, secondary cancers are a key problem with chronic immunosuppression, and for type 1 diabetes therapies currently in development the potential extent of this problem is not known. Hence, every effort possible should be made to monitor Epstein-Barr virus, herpes simplex virus, and cytomegalovirus reactivation in ongoing immunosuppression trials. Over the long-term, studies of safety should also determine the likelihood of other adverse effects, especially those of renal and cardiovascular origin, given their intimate relationship to sites for type 1 diabetes-associated complications. Indeed, an ethical examination of the fine balance between acceptable side effects and efficacy remains

a key issue for any new therapy. Aside from issues of safety, additional studies should evaluate whether the preservation of beta cell function in recently diagnosed type 1 diabetes patients offers short- and long-term clinical benefit with respect to disease-associated complications, particularly those of retinopathy, nephropathy, and neuropathy.

- **Enhance animal models for the study of relevant immune mechanisms and potential interventions.**

Risks associated with testing interventions in human clinical studies, plus recent advances in animal models, provide ample justification for accelerating development of animal models to study human type 1 diabetes-relevant immune processes and potential interventions. For example, newly derived mouse models with greater fidelity to disease (genetically engineered or transplanted with human molecules and tissues) should be given priority testing for their ability to serve as human surrogates for investigation of therapies aimed at attenuating anti-beta cell immunity. Again, such models are common means to the ends outlined not only for Goal II described here, but also for Goal I (the evaluation of the human genetic and environmental risk factors) and Goal III (the evaluation of methods and mechanisms relevant to islet transplantation).

Research Objective—Develop a Safe and Universal Means for the Primary Prevention of Type 1 Diabetes:

- **Further investigate the potential utility of autoantigens as "vaccines" for prevention of anti-beta cell autoimmunity.**

It is possible that future research will show that altering or knocking out islet autoantigens will abrogate islet autoimmunity in animal models such as the NOD mouse, as was the case for insulin. If so, this finding would support the notion that type 1 diabetes can depend on more than one autoantigen, as suggested by the existence of multiple autoantibody and T cell specificities in affected NOD mice. Timing could be important here as well. For example, certain "self" targets may be prominent only in some earlier (or later) stages of disease progression. Such possibilities, currently being intensively researched, are expected to provide information that will be critical for the design of effective autoantigen-based vaccination strategies. Furthermore, it is possible that innovative therapies such as vaccines capable of preventing type 1 diabetes could be developed without the identification of specific environmental targets or beta cell autoantigens for type 1 diabetes. Thus, vaccination against other, even causally unrelated agents may, through modulation of the immune response, confer protection against type 1 diabetes. Although efforts directed at such approaches have not been fruitful to date, this remains a potentially valuable area for further research.

- **Complete enrollment into the TRIGR study and begin analysis of the importance of exposure to cow's milk protein in the development of islet autoimmunity and type 1 diabetes.**

The immature intestine allows leakage of undigested dietary proteins, which may be antigenic. Although the causes of diabetic autoimmunity in humans remain controversial, studies in diabetes-prone mice and rats show that hypo-antigenic weaning diets are protective. TRIGR seeks to determine whether the risk of type 1 diabetes is different in genetically susceptible infants who are weaned onto a hydrolysate of cow's milk formula, in

which many of the cow proteins have been broken down, versus standard cow's milk formula. In addition to answering this important question, the study includes a series of mechanistic studies that will be conducted among children participating in TRIGR. These studies will complement TEDDY in addressing the possible role of enteroviral infections, dietary factors and gene-environment interactions that may provide the basis for future clinical trials.

- **Begin the design and implementation of clinical trials aimed at reducing the impact of environmental factors that trigger islet autoimmunity and type 1 diabetes *in utero*, during early postnatal life, and later development.**

Many studies (e.g., T1DGC, TEDDY, TrialNet Natural History Study, and TRIGR) are accumulating vast amounts of data and samples that can be used to better define genotypes and phenotypes in patients with type 1 diabetes and their family members. These data will be important for designing and implementing clinical trials for the translation of study findings, for example, through the TrialNet clinical trials infrastructure. Identification of potential triggers through epidemiological studies could directly lead to the design of clinical trials. For example, if confirmed in other ongoing studies, suggestive data about preventing type 1 diabetes by elimination/modification of exposure to cereals might be the basis of a future clinical trial. Successful prevention strategies could ultimately be implemented in the general population.

Table 2. Key Research Objectives for the Prevention and Reversal of Type 1 Diabetes

- Identify and Optimize the Detection of Immunologic, Genetic and Metabolic Markers of Type 1 Diabetes
- Understand the Interplay Between Early Environmental Encounters and the Immunoregulatory Defects, Which Results in Beta Cell Destruction in Human Type 1 Diabetes
- Advance Basic Understanding of Facets of the Immune Response (e.g., regulatory T cells, innate immunity, etc.) that Have Recently Been Appreciated as Key Mediators of Beta Cell Destruction
- Identify an Intervention Capable of Long-term Reversal of Recent Onset Type 1 Diabetes without Concomitant Short- or Long-term Adverse Effects
- Develop a Safe and Universal Means for the Primary Prevention of Type 1 Diabetes

GOAL III: DEVELOP CELL REPLACEMENT THERAPY

Why This Goal Is Important to People

Those who live with type 1 diabetes ask: "Will there be a cure for my disease? If cell therapy becomes available, will I be eligible? Are there side effects? How are scientists working to solve the problem of finding a source of insulin-producing beta cells so that all patients can someday receive an islet transplant?"

The prospects of a cure for type 1 diabetes have improved dramatically because of research advances in cell-based therapies. Using improved techniques, researchers are now capable of restoring substantial insulin production to type 1 diabetes patients for a period of a few years by transplanting clusters of insulin-secreting beta cells. These cell clusters, called islets, are obtained from the pancreatic tissue of human organ donors. The transplanted cells are functional replacements for those destroyed by the disease through a misguided attack by the body's own immune defense system (autoimmunity). Unfortunately, this experimental procedure--called islet transplantation--is still limited for several reasons. First, there is not an adequate supply of donor tissue to treat all type 1 diabetes patients who might benefit from this procedure if it were to become widely available in medical practice. Second, patients who receive islet transplants require lifelong medication to keep their immune systems from rejecting the new cells as "foreign." This regimen of immunosuppressive drugs has many unwanted side effects and is a major barrier that currently permits the study of islet transplantation only in adults with brittle diabetes or who already receive immunosuppressive drugs after kidney transplantation. If these and other barriers can be overcome, the standard of practice for treating type 1 diabetes could be revolutionized. Achieving the goal of developing safe cell-based therapy would dramatically improve the health and quality-of-life of type 1diabetes patients. NIH-supported research is critically important for meeting these research challenges.

Benefits of Cell Replacement Therapy

Although research advances have improved the management of type 1 diabetes, patients often have difficulty controlling their disease. No matter how vigilant patients are, they cannot achieve the exquisite regulation of blood glucose levels that is provided by a healthy pancreas. When the body's blood glucose level is not properly balanced, health complications of diabetes arise sooner and have more devastating effects over time (if sugar is too high), or an individual can become nervous, shaky, and confused, judgment can be impaired, and fainting and even death can occur (if sugar is too low). Therefore, researchers are working on ways for patients to improve control and avoid these complications. Replacing the insulin-producing pancreatic beta cells that have been destroyed by the disease would enable the body to assume its normal role of precisely regulating blood glucose levels. Patients would no longer have to perform "finger sticks" to check their blood glucose levels; inject themselves with insulin; worry about when to eat their next meal; or be plagued by the fear of life-threatening bouts of dangerously low blood glucose (hypoglycemia). Furthermore, islet transplantation can achieve the tightly regulated

blood glucose control that has been shown to slow or prevent the development of long-term disease complications. In short, realizing this goal would enable patients to live a life free of the everyday burden of this disease and to be spared from developing life-threatening disease complications.

Significant progress in islet transplantation has been achieved in recent years. Several research centers have shown, on a modest scale, that people with type 1 diabetes who receive transplanted islets can remain free of insulin injections for substantial periods of time. However, major challenges must be overcome before large-scale implementation of islet transplantation will be feasible. First, the methods of acquisition and delivery of islets must be optimized in order to provide replacement therapy for all people suffering from type 1 diabetes. This includes refining the islet transplantation procedure to avoid complications such as bleeding. Second, clinical treatments must be developed that will better combat the body's tendency to destroy transplanted islets. Finally, the mechanisms of pancreatic beta cell development must be elucidated to facilitate methods for producing cells in sufficient quantities to provide an adequate supply for transplantation.

Improving Islet Transplantation Techniques and Overcoming Challenges

In current methods of islet transplantation, insulin-producing cells are taken from a donor human pancreas and transferred, or "grafted," into an adult patient, most commonly in the liver. Once implanted, these grafts begin to make and release insulin in response to the body's needs. The transplanted cells thus enable the patient's efficient use of sugar for energy, and keep the level of sugar in the blood finely balanced. The goal is to transplant a sufficient quantity of insulin-producing cells to keep the blood glucose level as close to normal as possible—with little or no reliance on external insulin administration.

Researchers have confirmed that islet transplant recipients are able to maintain near normal blood glucose levels, which is the primary and most highly desired outcome. They also have observed, however, that success of the transplantation process varies greatly and wanes over time, underscoring the need for further research on methods of obtaining islets for transplantation and maintaining functioning transplanted islets. Progress has also been made in development of laboratory tests to assure that high-quality islet cells are used for transplantation, and in refinement of the technique for implanting donor islets into patients—both of which are key to optimizing the success of the treatment.

Limitations remain for the expansion of the islet transplant technique. For example, for the many steps that occur prior to the transplant surgery, the fragile islet cells must be collected and handled very carefully so as to preserve their health and function. Improper handling of cells renders them of little benefit to the patient. Likewise, these healthy donor cells must be implanted into the patient in an environment that continues to promote good health and function. Many of the complex details of what constitutes this type of environment are not yet completely defined. Scientists are investigating alternative surgical sites, as well as sophisticated biomaterials that may protect the islet graft from destruction by the immune system. Improving the collection and transplantation methods for islets is crucial for immediate expansion of the

technology. The greater the survival of cells, the lower the number of cells required, and thus the greater the number of patients who can undergo this life-altering treatment. To reach the goal of improving islet transplantation, future research will need to focus on: improving the processing and handling of islets; developing techniques to measure viability and predict success of the islets prior to transplant; and developing and refining islet transplant techniques.

Fighting the Immune System's Rejection of Transplanted Islets

Patients who undergo islet transplantation are required to take lifelong medications to prevent the immune system from attacking and destroying the transplanted cells. However, these drugs can cause serious and adverse side effects, can reduce the body's ability to fight infections, and also may weaken or kill the grafted cells. Researchers are gaining a deeper understanding of the concept of graft rejection and how to identify early signs of rejection, at a point when intervention is possible. They have developed new, less toxic agents to block the immune attack on the transplanted islets. These agents are close to being tested in a limited number of islet transplant recipients. In these pilot clinical trials, researchers will study new approaches to help to ensure that all patients who undergo treatment have the greatest opportunity to achieve successful results. In order to reach the goal of reducing immune rejection and recurrent autoimmunity, future research should focus on developing novel immunomodulation strategies and technologies, and on creating techniques capable of monitoring and preventing autoimmunity and rejection.

Understanding Beta Cell Development and Function

A major restriction of islet transplantation is tissue supply, which is currently limited to donor pancreatic tissue. However, research is under way to develop methods for restoring insulin production by regenerating beta cells, or by producing beta cells generated from stem/progenitor cells. If successful, these methods could lessen or obviate the reliance on donor pancreatic tissue as a source of transplantable cells.

Researchers have accumulated considerable knowledge about the basic biology of pancreatic beta cells, both in terms of how these cells function and how they are affected in type 1 diabetes. Methods have been developed to study the genes that are uniquely active in beta cells, and the proteins those genes produce. Knowledge is expanding about stem/progenitor cells that differentiate into insulin-producing beta cells. Studies have suggested that it may be possible to coax the small number of insulin-producing cells that might remain in individuals with type 1 diabetes to multiply and once again produce insulin. Studies are also shedding light on the mechanisms whereby islet cells are damaged in diabetes; on ways to monitor the status of transplanted islets; and on possibilities for regenerating beta cells or for boosting residual beta cell function.

Future research will clarify currently unknown aspects of the body's insulin-producing beta cells. Much remains to be discovered about these cells, as well as the stem cells from which they are derived. Specifically, the genes, proteins and other biological compounds associated with beta cell development must be elucidated. Furthering basic research on these vital cells will greatly enhance efforts to produce an abundant supply of them for transplantation. The potential

outcomes of these studies would permit scientists to grow islet cells for use in future research efforts. This knowledge would also help scientists recreate an environment in the transplant patient that would optimize the success of the grafted islets as well as make the treatment more widely available. In order to understand pancreatic beta cell development, avenues of future research should include efforts to identify and understand the workings of the genes that participate in development of the pancreas; to develop pancreatic beta cell lines and animal models for use in research and transplantation; and to comprehend the signals and processes required for maintenance and expansion of beta cells.

Islet transplantation is a promising therapy that can yield long-lasting and beneficial results for individuals with type 1 diabetes. Significant progress has been made in expanding the knowledge of islet cell biology and the processes associated with transplantation and immune rejection. The clinical strategy remains limited, however, due largely to constraints in islet supply and the consequences of immune rejection. Future research is needed to make islet transplantation a medical alternative for all individuals suffering from type 1 diabetes. Addressing islet transplantation, immune rejection, and beta cell development will permit researchers to move closer to a cure.

GOAL III: DEVELOP CELL REPLACEMENT THERAPY

INTRODUCTION AND BACKGROUND

Despite compliance with a regimen of insulin replacement therapy, patients with type 1 diabetes do not achieve normally regulated metabolism. Efforts to continuously maintain blood glucose within the normal range often result in episodes of severe hypoglycemia that can manifest debilitating consequences including unconsciousness. Islet transplantation has been investigated as a treatment for type 1 diabetes, particularly in these labile patients. The simplest measures for success have been insulin independence and near normalization of metabolic control with ability to reduce HbA1c levels below 6.5 percent, in the absence of severe hypoglycemia. Refinement of islet preparation technologies and immunosuppression strategies has dramatically advanced clinical islet transplantation, creating a potential new curative paradigm for the disease. Islet transplantation research has witnessed resurgence, new centers have emerged, and the ability to achieve post-transplant insulin independence has been validated worldwide.

Although the advances described in this chapter have brought tremendous hope for a cure, they have also brought realization of formidable obstacles limiting more expanded applications of islet cell transplantation. First, there is a grossly inadequate supply of donor pancreata for the number of potential recipients and a need for improved methods to produce islets more efficiently and predictably. Most commonly, islets are obtained from donor cadaveric pancreata that may not always be functionally adequate for transplantation. The transplantable islet mass recovered from a single pancreas rarely suffices to completely reverse diabetes, and insulin-independence typically requires islets obtained from multiple donors. Therefore, researchers are seeking ways to optimize organ procurement and the islet isolation process from these precious and finite resources. Consonant with these efforts, research is in progress to determine if islets obtained from human pancreata can be coaxed into producing more insulin producing beta cells, and whether a progenitor/stem cell[2] can be identified that could be converted into a beta cell. Alternative sources to human islets are also in experimental investigation, from studies using genetically modified pancreatic cells and using surrogate porcine islets that may extend the numbers of potential beneficiaries. While type 1 diabetes results from an immune attack specifically targeted against the islet's insulin producing beta cells, growing evidence suggests that this autoimmune process may not completely destroy all insulin-producing cells and that surviving cells may have the potential to recover and regenerate under appropriate conditions. Thus with further research, the sourcing barrier may be overcome not only by producing renewable supplies of transplantable beta cells, but also by healing islets and regenerating the lost beta cells within the diabetic pancreas and the graft. Importantly, achieving this goal requires increased knowledge about the underlying molecular mechanisms that control beta cell development, growth and function.

The current requirement for lifelong immunosuppression following islet transplantation is another challenge that limits the widespread use of this treatment option for type 1 diabetes. Drug intervention is needed not only to prevent rejection of transplanted islets, but also to prevent recurrence of the underlying autoimmunity that initiated the disease. Because most of

[2] The NIH supports human embryonic stem cell research consistent with federal funding policies.

the potent immunosuppressive drugs also carry toxicities, the balance between benefit and risk must be assessed to determine the most appropriate patients for treatment. The risks are such that, despite the burdens of imperfect methods to control blood glucose, islet transplantation is currently offered only to type 1 diabetes patients who suffer from recurrent hypoglycemia (see Goal IV) or who are kidney transplant recipients. Regrettably, this barrier also currently prevents the use of islet transplantation as a viable treatment strategy in children. Research to identify less toxic methods to prevent islet rejection and the recurrence of autoimmunity is crucial to realize the promise of islet transplantation for type 1 diabetes patients.

Cell replacement therapy can eliminate the need for the endless finger sticks, needles, and anxiety currently endured each day by type 1 diabetes patients. To make the widespread use of this therapy a reality, it is imperative to promote research in both basic islet cell biology, in order to understand beta cell development and growth, as well as in islet transplantation. It is also critical to develop strategies that will permit long-term post-transplant function of the insulin producing cells without the need for life-long recipient immunosuppression.

RECENT RESEARCH ADVANCES

Islet Transplantation

Recent advances in the field of islet transplantation have held out the hope of a turning point in the treatment of type 1 diabetes. Within the past five years, it was demonstrated that transplantation of human pancreatic islets can reproducibly reverse hyperglycemia and often result in insulin independence. These dramatic results have been obtained in some instances using only a single donor pancreas. Transplantation was attempted in adult type 1 diabetes patients whose diabetes was labile despite intensive efforts to correct inappropriate blood glucose levels with insulin. The islet transplant corrected hypoglycemia unawareness, virtually eliminating the occurrence of debilitating severe hypoglycemic episodes. These impressive findings have been confirmed by independent centers in North America and Europe, and through the Collaborative Islet Transplant Registry (CITR), which was established and funded by NIH to compile and analyze the aggregate clinical data from many of these centers. Through such analyses, islet transplantation is clearly shown now to be a viable therapeutic alternative for some patients. However, variability in islet function is encountered immediately post-transplant, and progressive loss of function of transplanted islets over time is often observed. These limitations underscore the need for continued development of improved islet procurement technologies and patient therapies needed to sustain the graft. The number of transplant centers performing clinical islet transplantation continues to increase, and insulin independence without hypoglycemic episodes is now an achievable goal. These advances bring heightened excitement to islet transplantation research and a greater appreciation of the challenges that must be resolved to extend the applications of this therapy.

Advances in Pancreas Shipment, Pancreas Preservation, and Islet Cell Processing Technologies: Pilot clinical trials have demonstrated that insulin independence and long-term islet graft function could be obtained not only with islets processed and transplanted at the same institution, but also with islets processed at regional NIH funded Islet Cell Resource Centers (ICRs) and shipped for transplantation at remote institutions across the U.S. This success has

validated the concept that regional centers could be utilized for islet cell processing and distribution. Furthermore, the establishment of ICR Centers has enabled an infrastructure that permits collaborative optimization of pancreas shipping devices, preservation media, islet isolation technology and interim storage through comparative assessments. The ICRs provide resources, structure, and a coordinated community of investigators with a focus on enhancing the quality of isolated islets, promoting basic islet research, and enabling additional facilities to perform the procedures. The ICR Centers work closely with the CITR so that data on islet procurement and production, as well as on clinical outcomes following transplantation in North America, are collated and disseminated. This joint effort facilitates comparative analysis that will ultimately determine the most effective and safe clinical protocols.

Assessment of Pre-Transplant Islet Cell Function: Significant progress has been made towards the development of quality-control tests to determine islet cell viability and function before transplantation. The ability of transplanted beta cells to restore and maintain normal glucose levels in the body is proportional to their relative mass and functional capacity. Therefore, the development of quality-control tests permits researchers to identify and discard non-functional or damaged islet cell preparations prior to transplantation. Such assessments minimize unnecessary exposure of patients to islet grafts unlikely to survive immunosuppressive and procedure-related risks.

Islet Transplantation Technologies: Following an extensive process of isolation from the donor pancreas, islets are infused via catheter into the portal vein and released into the recipient's liver**.** In a successful transplant, the islets become embedded in the liver and produce insulin in response to metabolic requirements. However, the catheterization and infusion of islets carry a risk of hepatic bleeding. Recent progress in islet infusion techniques--with the introduction of gravity infusion bags and other improved technology--has reduced the risk of bleeding after catheterization and also minimized episodes of obstruction of the branches of the portal vein.

Immunotherapies and Immunosuppression Pipeline: Islet transplant recipients must endure a lifelong regimen of immunosuppressive drug therapy to prevent their immune systems from destroying the transplanted cells. These maintenance regimens can have serious, unwanted side effects, and can also be directly toxic to the islet beta cells. Recently, significant progress has been made in the development of novel and less adverse immunomodulatory agents. These new therapeutic agents may enable more effective modulation of the recipient immune response, while also decreasing the side effects associated with chronic administration of currently available anti-rejection drugs. Some new agents studied in other diseases and/or animal models are already moving into pilot clinical trials of islet transplantation. A further pipeline of novel molecules and drugs is currently under development, or already at the pre-clinical level of testing, and may soon become available for pilot clinical trials of islet transplantation.

Induction of Immune Tolerance: As described in the preceding chapters, the immune system has the complex task of recognizing foreign molecules while disregarding molecules native to the host ("tolerance"). Current immunology research has shown that the activation of the immune response appears to require an intricate pathway or network of molecular signals, some of which are key for discriminating between foreign and self molecules, the so-called "co-stimulatory" signals. Other mechanisms for self-tolerance include elimination of self-reactive T

cells or antibodies, or induction of regulatory cells that can actively suppress autoimmunity. Understanding immune activation in animals has enabled researchers to target specific steps in these pathways for therapeutic intervention to either suppress active immune cells or achieve tolerance to foreign tissues. Long–term islet graft survival in animals without the requirement for long-term immunosuppression has been demonstrated using methods that suppress co-stimulatory signals to immune cells. The NIH has placed a high priority on facilitating innovative research on mechanisms of immune tolerance in individual research projects and in cooperative study groups. For example, support has been provided for pre-clinical studies in the Non-Human Primate Transplantation Tolerance Cooperative Study Group (NHPCSG) and for clinical studies in the Immune Tolerance Network (ITN) and the Clinical Islet Transplantation Consortium (CIT). The success witnessed in animals that show sustained islet survival and reversal of diabetes after discontinuation of all anti-rejection drugs suggests that tolerance may also be achievable in humans. Monoclonal antibodies, other blocking agents, and selected cytokine combinations are under intense investigation to prevent immune activation and induce immune tolerance in islet transplantation. Identification of such novel therapeutic agents can help to overcome the current barrier imposed by the requirement for continuous immunosuppressive therapy after transplantation. As described in previous chapters, these therapeutic agents may also have added utility for preventing or reversing the initial autoimmune process.

Immune Monitoring for Early Diagnosis of Rejection and Tolerance: Unlike the scenario for solid organ transplants, there is no biochemical marker for islet rejection that enables detection of islet loss early enough following transplantation to permit effective intervention and rescue. At the time of documented hyperglycemia and need for return to exogenous insulin administration, significant islet loss has already occurred. This observation is similar to the situation that occurs at the onset of type 1 diabetes, as described in the previous chapters. Scientists have recently demonstrated elevated expression of several key genes in the peripheral blood associated with inflammation--an event that precedes clinical evidence of post-transplant islet loss. Such gene expression profiles may serve as molecular signatures that foretell graft rejection. In addition, this information may also provide predictive guideposts for withdrawal of immunosuppression. The early detection of these destructive events will enable development of effective intervention strategies to reverse immune activation after islet transplantation, before islet cell destruction occurs.

Islet Cell Biology

Many scientific advances over the past five years have markedly advanced the understanding of how pancreatic beta cells develop and function and how they are adversely affected in type 1 diabetes. These advances have been due largely to new information flowing from completion of the human and mouse genome sequencing projects, and the development of novel technologies that have enabled researchers to study the genes expressed in the pancreas--both in humans and in a variety of model organisms.

New Technology To Study Developmental Biology of the Endocrine Pancreas: A few key genes code for special transcription factor proteins that regulate the expression of many other genes. To understand the necessary steps for a progenitor/stem cell to develop into a beta cell, it

is important to identify the transcription factors and the downstream target genes that mediate this transition. A major barrier to determining these steps is that these progenitor cells are transient in nature and found in vanishingly small numbers. Furthermore, no methods exist to prospectively isolate purified populations of pancreatic progenitor/stem cell populations. Recently, scientists have created mouse models that allow researchers to visually track the expression of transcription factors that characterize pancreatic progenitors at various stages of progression toward mature beta cells. Using these genetically engineered mice, researchers could isolate pancreatic beta cells by an experimental technique called "fluorescence activated cell sorting (FACS)." This advance yields pure populations of mouse pancreatic beta cells, which can then be used to gain further insights into which genes regulate beta cell development and function. Importantly, through this approach, researchers will be able to identify appropriate cell surface markers on pancreatic progenitor cells. Pursuit of this research avenue could pave the way to the isolation and prospective purification of human progenitor cell populations.

Role of Master Control Genes in Regulating Formation of Pancreatic Beta Cells: Researchers have identified important transcription factors, which are proteins that turn genes "on" and "off," that have essential roles in either the formation or function of the pancreas, pancreatic islets, or pancreatic beta cells. Some of the transcriptional regulators expressed in pancreatic beta cells during development, when mutated, have been found to cause rare forms of diabetes mellitus termed *Maturity Onset Diabetes of the Young (MODY)*. Identification of many of these transcription factors was the result of years of systematic studies of the insulin promoter, the part of the insulin gene that regulates its expression. This research pinpointed specific regulatory DNA sequences within the promoter and the transcription factor proteins that bind to them. Other key genes were serendipitously discovered. For instance, the gene neurogenin 3 (*ngn 3*), which was being studied for a possible role in brain development, was found to be essential for the formation of pancreatic islets, including beta cells. The identification and molecular characterization of key transcription factors, such as *Pdx-1*, a master regulator for formation of the endocrine pancreas and a *MODY* gene, provides a starting point for understanding the complex gene regulatory networks that exist within the pancreatic progenitor cells, and well as the mature beta cells. These studies can help researchers identify the necessary steps to turn progenitor/stem cells into insulin-producing beta cells.

Recognition of the Regeneration Potential of Pancreatic Beta Cells: Evidence has been gained over the past five years indicating that both humans and animals have some ability to regenerate beta cells. Thus, it may be possible to restore beta cell mass in type 1 diabetes patients whose beta cells are not completely destroyed. Many tissues have been found to contain progenitor/stem cells that could restore lost cell types. However, it is not yet clear whether such a cell type exists in the pancreas, and whether it can form new beta cells after all existing beta cells have been damaged or destroyed. Recent studies in mice imply that the proliferation of new beta cells after injury contributes predominantly to the new beta cell population. This insight has contradicted older models of pancreas regeneration. The novel findings need to be expanded to enhance understanding of the regenerative potential of beta cells, and determine whether beta cell proliferation is a clinically significant process. Such studies are essential because, even if the number of residual beta cells is small, perhaps only a few cells may be needed to generate sufficient number of cells to restore lost beta cell function in a type 1 diabetes patient.

Steps Toward the Creation of New Beta Cells from Stem Cells: In 1998, researchers reported deriving the very first human embryonic stem (ES) cells. Since then, the potential of inducing human ES cells to form a wide variety of other cell types has been demonstrated by several groups of researchers. With these remarkable advances, the possibility has emerged of generating from human ES cells large amounts of either pancreatic beta cells, or whole pancreatic islets. However, several barriers have become apparent as a result of attempts to convert human ES cells into insulin secreting beta cells. The major barrier is the lack of knowledge about how to direct the differentiation of ES cells, or any other progenitor/stem cell type, towards a pancreatic beta cell fate. It has become clear that ES cells, or any other starting cell type, may need to be induced to pass through many intermediate cell fates, just as occurs when pancreatic beta cells are formed during development. In contrast to the insufficient number of cadaveric donor pancreata for use in islet transplantation procedures, this avenue of research has the potential to create an unlimited supply of islets for transplantation.

Imaging the Pancreatic Islet: Since 1999, there has been significant progress toward directly visualizing the pancreatic beta cells, transplanted islets, and the inflammation of type 1 diabetes using imaging technologies, particularly Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) (see Goal VI). Isolated human islets have been labeled with non-toxic imaging agents that allow them to be seen after transplantation into animals. Targeting molecules that can carry imaging agents directly to proteins on the beta cell surface are being developed to permit counting the number of beta cells in people. When the pancreas is under attack by the immune system, its blood vessels become “leaky;” this process can be visualized by an imaging molecule that moves from the blood into the inflamed tissue. This ability to actually see the cells and the processes associated with disease would help researchers better understand the life-cycle of the islet and how it is damaged in diabetes, and also monitor therapy in patients.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

Islet Transplantation

The overall clinical experience in islet transplantation has highlighted the complexity of a sequential approach that can last days between pancreas procurement, islet processing, pre-transplant culture, recipient immune-conditioning, and the final islet infusion. To maximize the use of scarce donor islets and minimize the risk of procedure-related complications, highly specialized and multidisciplinary teams are required for success. It is necessary not only to obtain uniformly high-quality islet cell products, but also to ensure optimal islet infusion techniques, infusion sites, and effective post-transplant patient management. A major barrier to transplantation is the immune response of the recipient against the donor islets. While current protocols achieve remarkable success in achieving insulin independence and/or improved glucose control without hypoglycemia, longer term follow-up suggests that this success wanes over time. Chronic rejection and recurrent autoimmunity are major challenges to be overcome in this regard. The following research objectives are critically important for a stepwise, integrated approach to develop successful cell replacement strategies to treat diabetes.

Research Objective—Develop Novel Strategies and Infrastructure that Support Advancing Pancreas Procurement and Islet Processing:

- **Study potential donor interventions that minimize the negative effects of brain death and ischemia (low blood supply)/hypoxia (low oxygen) on islet survival and function.**
- **Develop improved preservation medium, shipping containers, and monitoring technology to improve pancreas preservation during transport.**
- **Develop improved islet isolation and purification methods and novel methods for tissue processing, beyond the currently available enzyme-blend techniques.**
- **Develop new strategies to improve pre-transplant islet culture that will sustain graft survival and function.**

Prior to transplantation, islet grafts have been exposed to a series of adverse conditions that sequentially contribute to a progressive reduction of the original islet cell mass. This loss of insulin producing tissue results from the collective incremental effects of many factors, including the molecular effects of donor injuries preceding islet isolation associated with brain-death and hypoxia; prolonged time of pancreas cold preservation and shipment to the islet cell processing center; enzyme-based tissue digestion techniques; and islet purification steps and pre-transplant culture/shipment to the final transplant facility. Improvement at all phases of these processes and technologies will be critically important to minimize islet loss and maximize the potential use of each donor pancreas. For example, potential strategies could include the delivery of anti-inflammatory agents and/or agents that enable survival of the pancreatic islets prior to procurement. These improvements may ultimately allow transplantation of sufficient numbers of islets obtained from only a segment of donor pancreas, such as in the case of living-donor islet transplantation.

Research Objective—Develop Improved Methods To Assess Islet Beta Cell Viability and Potency that Predict Early Islet Function after Transplant:

- **Define and implement novel strategies and methods for assessment of beta cell-specific viability and function.**
- **Develop predictive tests to determine the suitability of an islet cell product for clinical use (i.e., tests predictive of post-transplant survival and function).**

To predict transplant success, assays to assess islet quality are needed that report on beta-cell-specific traits, rather than general traits like oxygen consumption. Such assays are needed because the ratio of beta cells to other pancreatic cells can vary greatly among preparations, and robust pancreatic exocrine cells in a partially purified islet preparation can easily utilize enough oxygen to mask damaged beta cells. The NIH has established Islet Cell Resource Centers (ICRs) across the country to provide high-quality human islets for treatment of type 1 diabetes, and for basic research. These Centers share a mission to improve the quantity and quality of available human islet tissue. The ICRs have been pivotal in establishing some uniform measures of "potency" testing of islets such as glucose stimulated insulin release, but research would benefit from new surrogate markers of islet quality, which are beta cell specific and correlate well with engraftment potential. Toward this end, islet teams that include cell biologists should work together with clinicians to establish and standardize potency testing, and to identify islet factors that predict success or failure in achieving insulin independence. Candidate assays include apoptosis markers and measures of cell function, measures of beta cell antigenicity, as well as characterization

based on proteomic or genomic technologies. An adequate measure of potency would correlate well with the ability of the islets to restore insulin independence in an appropriate animal model (e.g., NOD-scid mice) and in human transplantation. Once such tests are established, it will be important to identify those factors in the islets that contribute to engraftment success and failure.

Research Objective—Improve Islet Transplant Procedures:

- **Determine the optimal sites for islet transplantation.**
- **Develop novel islet survival strategies.**

Currently, the liver is the preferred site for islet transplantation, which has allowed for excellent initial islet survival and function. However, islets that are transplanted into the portal vein of the liver are in suboptimal or even hostile quarters, and the liver site may contribute to limited islet longevity. After infusion into the liver and upon entering the blood stream, islets are immediately exposed to a chemical assault. For example, islets embedded in the liver are exposed to above normal amounts of metabolic toxins and are subjected to high levels of toxic immunosuppressive drugs. Prior to engraftment, islets must also survive without the assistance of an intact network of blood vessels (as contrasted with whole organ transplants). Therefore, novel approaches are needed to ensure engraftment immediately after transplant, as well as increase the lifetime of the graft. Islets transplanted into the liver have been shown to lose their ability to respond to hypoglycemia (termed a "counter-regulatory response"). This impaired response is not seen when the islets are placed in other sites. Furthermore, changes in liver structure have been recently described following some cases of islet transplantation. Possible alternatives to the liver site at minimum include the spleen, omentum, pancreas, and muscle. Several of these alternative sites would have the added advantage of providing retrievable and replaceable grafts. Preventing early post-transplant islet loss remains a challenge, but is critical to graft survival.

Research Objective—Develop Novel Methods To Accurately Assess the Post-Transplant Islet Mass:

- **Define and implement post-transplant metabolic testing of the transplant recipients to estimate functional islet mass that successfully engrafted, and estimate eventual changes in functional islet mass in long-term post-transplants.**
- **Develop novel strategies for imaging of islet cells post-transplant and/or in the native pancreas (PET, MRI, video-endoscopy, *in vivo* microscopy, etc).**

It is essential to develop novel methods to accurately assess the mass of insulin producing tissue that successfully engrafts post-islet transplantation. Both metabolic and imaging strategies are needed to define islet survival during the early post-transplant period as compared to the islet mass initially infused. These methods could permit monitoring of long-term changes in islet mass not only at the transplant site, but also in the pancreas of the patient, to assess, for example, the effect of therapeutic strategies on beta cell regeneration in the native pancreas. Achieving this objective will require collaboration among physicians, imaging experts, chemists, and biologists and is discussed more fully under Goal VI.

Research Objective— Harness New Understanding of the Immune System To Develop Improved Clinical Monitoring and Immunotherapies:

- **Identify markers of immune rejection and recurrent autoimmunity.**
- **Define effective strategies for immunomodulation of the recipient immune response and for tolerance induction following islet transplantation.**
- **Develop effective strategies for T lymphocyte regulation.**
- **Develop novel strategies for co-stimulatory blockade and expansion of candidate humanized monoclonal antibodies for co-stimulatory blockade.**

There is a great need for biochemical markers, accessible in the peripheral blood, to enable the timely detection of islet loss; assess inflammation; and permit effective intervention and rescue. Research is warranted to assess lymphocyte gene expression in blood as a means to monitor rejection. It is also necessary for more discovery research to help develop other methods of monitoring early immune activation, such as non-invasive imaging before islet cell destruction occurs. As described in Goal II, promising areas of research include both cellular functional assays, as well as biomarkers based on broad measurements of gene expression, including proteomics. Such assessments could help provide the scientific rationale and guidance for the optimal time to adjust and eventually discontinue immune therapy. Novel strategies of immunomodulation have recently demonstrated in animals that islet transplantation could reverse diabetes without a continuous requirement for immunosuppression. Long-term survival of mismatched islets that produced 100 percent insulin independence with normal blood glucose levels was obtained even in diabetic non-human primates after a brief course of co-stimulatory blockade. If the success witnessed using these markedly reduced levels of toxic immunosuppression can be reproduced in diabetes patients with autoimmunity, clinical islet transplantation may ultimately provide a curative therapy. The brief exposure to immunosuppression is key because, as previously discussed in Goal II, risks that accompany continuous immunosuppression limit the numbers of potential beneficiaries for this procedure. Therefore, extension of translational research efforts is still required to define novel immune interventional strategies aimed at blocking co-stimulation. In addition, efforts must be expanded to understand relevant mechanisms of immune regulation, especially those that induce specific tolerance to the graft. Additionally, researchers should explore novel strategies such as those that exploit T lymphocyte regulation and the infusion of donor immune and progenitor cells to achieve tolerance. Tissue engineering strategies that incorporate materials or devices that keep the islets isolated from the immune system could also be enormously helpful in shielding islets from rejection. Collectively, these objectives represent overlapping aims also shared with the prevention or reversal of type 1 diabetes in its early stages.

Pancreatic Development, Stem Cells, and Regeneration

Furthering basic research in developmental and stem cell biology of the pancreas would greatly enhance efforts to produce an abundant supply of pancreatic beta cells for transplantation, or to restore to normal, the mass of damaged or destroyed beta cells in individuals with type 1 diabetes. Of paramount importance is creating and freely distributing reagents of particular usefulness to beta cell biology research, such as poly- and monoclonal antibodies and relevant animal models and cell lines, as well as applying tools of functional genomics and proteomics. These key reagents and resources are needed to accelerate research focused on identifying,

isolating, and differentiating islet-stem/progenitor cell populations to generate beta cells, as well as to propel research on understanding mechanisms of beta cell regeneration. The creation and utilization of these key resources will open new avenues towards the development of cell-based therapies in diabetes.

Research Objective—Make Pancreatic Beta Cells in Tissue Culture in the Laboratory:

- **Identify and characterize genes that play particularly critical roles in the formation of the pancreas.**

By understanding pancreatic development, researchers may be able to recapitulate normal development of beta cells in tissue culture using stem cells obtained from the patient or from other human donors. It will be necessary to perform more standardized and highly defined studies of both the gene and protein expression profiles of pancreatic beta cells and their progenitors during development. One strategy is the application of bioinformatics tools as they become available for modeling the sequential activation or repression of genes during pancreatic organogenesis. These studies should be directed at identifying, defining, and then characterizing the changes that occur in gene regulatory networks. It will also be necessary to perform detailed genetic studies to understand the functional importance of these regulatory genes in a mammalian model organism such as the mouse. While the mouse should remain the primary model system, other genetic model systems such as zebrafish could be exploited for discovering novel genes and pathways involved in pancreatic development. Characterizing the regulatory mechanisms that underlie the formation of the endocrine pancreas will provide the basis for understanding how to grow pancreatic islets in the laboratory. Another fundamental question to address is how newly forming beta cells acquire their antigenicity. Identifying and characterizing genes that play a role in this process will allow investigators to test whether type 1 diabetes is initiated by a defect in the beta cell--research that could provide new information about the etiology of type 1 diabetes.

- **Develop reagents and protocols for isolating pancreatic endocrine progenitor cells**.

The development of monoclonal antibodies to cell surface markers of beta cell precursor cells would allow the rapid isolation of target populations after embryonic stem (ES) cells have been induced to differentiate. To this end, key resources need to be developed, including establishment of a collection of mouse and human ES cell lines that are genetically tagged with markers that faithfully report the expression of genes that specify pancreatic progenitor cell types. Together, these reagents would enable the prospective isolation of progenitor intermediates, one of the first steps towards making pancreatic islets in culture.

- **Identify growth conditions that would permit the step-wise differentiation of beta cells from stem cells or precursor cells.**

A multitude of signaling pathways is active during formation of the endocrine pancreas. However, new research is needed to determine the right combination of growth factor signals and to perform high-throughput screens to identify novel small molecules that would enhance stage specific differentiation of the endocrine pancreas and expansion of progenitor cells. Accruing this missing knowledge would allow the creation of a protocol to both expand and differentiate cells, a key step in the development of a stem/progenitor cell-based replacement therapy.

- **Develop animal models to test the engraftment, survival, and metabolic impact of beta cells or islets derived in culture from stem/progenitor cells.**

Quantitative transplantation assays are needed for assessing the efficacy of cell replacement therapies. Mouse models that are engineered to accept human cells or non-human primate models for safety testing are of particular importance. The development of appropriate animal models for testing potential cell replacement therapies is a critical step before human therapies can be realized.

- **Determine if multipotent cells from fetal and adult tissue might be viable sources for beta cell replacement therapy.**

Many potential sources of cells might be used as a starting point for generating pancreatic islets. These sources could include cells from pig, human amniotic cells, and adult human multipotent cells isolated from bone marrow, liver, pancreas, or gut.

Research Objective—Understand How Mature Beta Cells are Maintained and Replenished in the Adult Pancreas:

- **Determine the mechanism by which beta cell number is restored after beta cell loss.**

A new challenge in beta cell research is to understand aspects of "islet maintenance," in particular, how an islet regulates its mass. Assessing beta cell mass and turnover quantitatively will require the development of better assays. The generation of monoclonal antibodies to cell surface markers on mature islet cell types, including beta cells, will aid in the development of new imaging methodology to assess beta cell mass *in vivo*. Researchers should investigate in animal models whether proliferation of pre-existing beta cells, or alternatively, the proliferation of other cells in the pancreas, contributes significantly to the formation of new beta cells. Understanding basic mechanisms of these processes is essential to developing strategies for the regeneration of any residual beta cells that may still exist in patients with type 1 diabetes, and may also provide clues for increasing the beta cell mass in those with type 2 diabetes.

- **Identify factors and agents for enhancing beta cell division or decreasing cellular apoptosis.**

Testing the ability of hormones and peptides to increase beta cell proliferation and/or induce autologous beta cell regeneration should be systematically explored in animal models, both in rodents and in large animal models such as pigs and non-human primates. High-throughput screens could be developed to identify combinations of growth factors or agents that could enhance beta cell growth. A project to develop immortalized human pancreatic beta cell lines that are functionally equivalent to primary beta cells has been initiated. The transplantation of human beta cells derived from these lines into appropriate type 1 diabetes animal models is an important first step towards the development of novel cell therapies. Investigators should determine why there are functional differences between isolated beta cells *versus* the beta cells located within a pancreatic islet. Understanding the basic mechanisms that regulate and maintain beta cell number and function could lead to strategies for preserving and/or restoring lost beta cells in those individuals whose cells have come under attack by their own immune systems.

Table 3. Key Research Objectives for Developing Cell Replacement Therapy

- Develop Novel Strategies and Infrastructure that Support Advancing Pancreas Procurement and Islet Processing
- Develop Improved Methods To Assess Islet Beta Cell Viability and Potency that Predict Early Islet Function after Transplant
- Improve Islet Transplant Procedures
- Develop Novel Methods To Accurately Assess the Post-Transplant Islet Mass
- Harness New Understanding of the Immune System To Develop Improved Clinical Monitoring and Immunotherapies
- Make Pancreatic Beta Cells in Tissue Culture in the Laboratory
- Understand How Mature Beta Cells are Maintained and Replenished in the Adult Pancreas

GOAL IV: PREVENT OR REDUCE HYPOGLYCEMIA IN TYPE 1 DIABETES

Why This Goal Is Important to People

Patients with type 1 diabetes walk a tightrope. Every time they check their blood glucose and adjust their insulin they must balance the immediate danger of low blood glucose and the long term risk of complications from high blood glucose. They worry "What if insulin therapy makes my blood glucose level drop dangerously low and I'm not even aware of it? Will I go into a life-threatening coma as a result? Who will even know this is happening and will they be able to help me?" Similar questions haunt the parents of young children with diabetes—many of whom cannot sleep at night because they are standing watch at their child's bedside. Facing an episode of dangerously low blood sugar—known as hypoglycemia—is one of the greatest fears of type 1 diabetes patients and their families. Thus, an urgent research goal is to find ways to prevent or reduce this acute danger of insulin treatment.

Insulin therapy is a life-saving treatment for people with type 1 diabetes. However, it is sometimes difficult for patients to gauge the exact amount of insulin that they should administer to themselves at any given moment to meet the needs of their body. Some patients use pumps that deliver insulin, but these devices have not yet been developed to the point where they can sense and respond to the body's need for insulin in an optimally calibrated way.

If there is too much insulin in the body, it increases the risk of hypoglycemia, or low blood sugar. When blood glucose levels fall below a minimal threshold, there can be serious and life-threatening consequences. Moreover, after a few episodes of hypoglycemia, patients with type 1 diabetes can lose the ability to sense drops in blood glucose levels, a problem called hypoglycemia unawareness. Therefore, they are at increased risk of additional hypoglycemic episodes. This risk is even greater at night, especially for children--causing anxious parents to go without sleep themselves so they can monitor their children for signs of low blood glucose levels. Adults with type 1 diabetes also worry about hypoglycemia unawareness. Those who have ever experienced severe low blood sugar episodes are especially fearful. Unfortunately, these fears can lead them to abandon a regimen of close control even though research has demonstrated its benefits in preventing or delaying the heart, eye, nerve, and kidney complications of diabetes. It can also limit their daily lives, making certain activities more hazardous. Clearly, research that leads to new or better ways to prevent or reduce low blood sugar could profoundly improve the health and well-being of people with type 1 diabetes, and their families.

Researchers are attacking the problem of low blood glucose from several angles. They need to understand how the body and brain normally communicate about blood glucose levels, and to identify what parts of this communication network are damaged or impaired in people with type

1 diabetes. They are asking several questions. How are a patient's awareness of impending low blood glucose levels and resulting hormonal responses impaired over time by recurring hypoglycemic episodes--a vicious cycle called "hypoglycemia-associated autonomic failure," or HAAF? How can a patient's brain adapt to protect itself from low blood glucose damage? What new clinical approaches would minimize the risk of low blood glucose, such as technologies that could better manage glucose level sensing and insulin delivery? An important tool for research in these critical areas will be animal models of disease. Through a multi-faceted approach, researchers hope to more rapidly achieve the ultimate goal of preventing or reducing hypoglycemia in patients with type 1 diabetes.

Sensing Low Blood Glucose

Understanding how the brain and body work together to determine and adjust blood glucose levels is a complex task, but researchers have made significant progress in the past decade. Scientists now know that glucose-sensing cells are stationed in key blood vessels to pick up signals and send them to the brain. They also know more about how those cells are "wired" to the brain so that it can receive continuous information about blood glucose levels. New insights are emerging about how the brain integrates this input with other metabolic signals it receives to develop the best response to keep blood glucose levels in a normal range.

Now that researchers have a "big picture," they are seeking the details of the body's sensing apparatus, and how it is affected by having type 1 diabetes. The underlying disease and insulin therapy may also alter the levels of other metabolic signals, thereby affecting how the brain responds to signals of low blood glucose levels. By identifying the mechanisms and other factors involved, researchers will be better positioned to develop effective medications to prevent precipitous drops in blood glucose in patients.

Preventing Hypoglycemia-Associated Autonomic Failure (HAAF)

What contributes to the recurring nature of hypoglycemic episodes and increasing unawareness that an episode is about to strike? Normally, a drop in blood glucose triggers the body's warning system to release stress hormones, including adrenaline, which results in symptoms such as shaking and sweating. These warning symptoms help make people aware that they need to eat or take other steps to increase their blood glucose levels. The body also reacts with "counter-regulation" defense measures, including the release of a hormone (glucagon) that elevates blood glucose. However, in people with type 1 diabetes, these alert mechanisms and defense measures are significantly impaired, and worsen with each episode of low blood glucose.

Being unaware of plummeting blood glucose levels is both dangerous and frightening. Moreover, patients are faced with the difficult fact that using insulin therapy to tightly control blood glucose levels and improve health in the long-term increases their risk of severe and worsening low blood glucose episodes in the short-term. Although the HAAF syndrome can be reversed by as little as several weeks of scrupulous avoidance of low blood glucose levels, it is difficult to accomplish this without losing good control of blood glucose levels--and thus losing the benefits of this control, such as preventing long-term complications. Thus, it is critically important to identify the as-yet unknown mechanisms responsible for hypoglycemia

unawareness so that new clinical strategies can be developed to combat HAAF, while simultaneously improving or maintaining good blood glucose control.

Future research will be focused on new strategies to prevent or reverse the HAAF syndrome. To do this, researchers will need to focus on three major objectives. First, they will need to identify how low blood glucose causes HAAF to develop, by studying this syndrome in animals. Animal models will also be important for research that can lead to preventive therapies. Second, researchers will need to use multiple tools to study what is happening in the brains and bodies of persons affected by HAAF and how it affects their management of diabetes. For example, brain imaging studies combined with hormone measurements could help researchers understand why patients are so vulnerable to hypoglycemia while they sleep. Finally, studies should focus on ways to restore the body's innate ability to counter low blood glucose levels with defense mechanisms that elevate blood glucose.

Protecting the Brain

The brain is almost entirely dependent upon blood glucose for the energy it needs to work. This dependency makes the brain quite vulnerable to episodes of low blood glucose. While it is crucial to discover ways to prevent these episodes in the first place, it is equally important to pursue therapies that patients can use to protect the brain from injury due to low blood glucose.

Developing effective new therapies will require knowledge of how the brain acts to obtain a constant fuel supply, and what it does to protect itself when its major fuel, glucose, is in short supply. Studies are providing insights about the alternate fuels and fuel reserves the brain uses when blood glucose is in short supply, and showing that patients with type 1 diabetes may use alternate fuels more efficiently. More is now known about how glucose and other fuels move from the blood into the brain. Now, emphasis needs to be placed on identifying the specific changes that occur in the brain in the face of low blood glucose and determining which of these are the most important to exploit for therapeutic intervention—both to reverse brain injury, and to promote protection from brain injury. For example, if use of alternate fuels is an effective way for the brain to protect itself from injury, then patients could take agents to increase those fuel supplies.

It appears that the brain's mechanisms for enhancing fuel efficiency and protecting itself from injury create a catch-22 situation: while protecting the brain from immediate injury, they seemingly "hide" low blood glucose levels from the patient, contributing to hypoglycemia unawareness. During a hypoglycemia episode, there is impairment in the patient's cognitive function—the higher-order brain processes like thinking and memory. However, it is not clear if there are long-term impairments once the episode is over. This picture is even more complex because insulin therapy itself may also directly affect cognitive function. Researchers are exploring these factors to better understand and prevent cognitive impairment in patients.

Building an Artificial Pancreas

Insulin therapy has improved tremendously over the past two decades, contributing to longer life and better quality-of-life for patients with type 1 diabetes. New forms of insulin, combined with

new technologies for blood glucose measurement and portable "pumps" for insulin delivery, have enhanced the ability of patients to control their blood glucose levels. Yet, current therapies to administer insulin are still an inadequate substitute for the body's own exquisite mechanisms for sensing and responding to insulin needs--mechanisms which are destroyed in type 1 diabetes. One major goal for research is to build an "artificial pancreas." Ideally, this would be a mechanical insulin-delivery system that could monitor a patient's blood glucose levels continuously, and would respond by releasing appropriate amounts of insulin, as needed, much the same way a healthy pancreas does. Such a system would spare patients from painful finger sticks to check glucose levels, and from administering insulin injections or monitoring an insulin pump. It would also greatly decrease the risk of severe low blood glucose episodes while improving glucose control, and thus reducing long-term complications. While development will require time and careful testing, researchers are rapidly exploiting technologies and tools necessary for such a system, including the very new methods for continuously monitoring blood glucose levels.

Severe hypoglycemia is an acute and potentially deadly risk of insulin therapy. Thus, until it is possible to prevent, reverse, or cure type 1 diabetes in medical practice, a high-priority research goal remains the development of better means of controlling and preventing low blood glucose episodes in patients whose lives are dependent upon insulin therapy.

GOAL IV: PREVENT OR REDUCE HYPOGLYCEMIA IN TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

Although insulin therapy is the cornerstone of type 1 diabetes management and prevention of disease complications, excessive treatment with insulin can result in hypoglycemia (low blood sugar levels). Too much insulin in the blood causes glucose levels to fall dangerously below a minimal threshold required to fuel the body's activities, particularly the brain. Even with newer forms of insulin that may decrease this risk, hypoglycemia remains an extremely serious life-threatening concern.

The potential for hypoglycemic episodes has limited the use of intensive insulin therapy protocols that are known to reduce the risk of longer-term diabetic complications, such as eye and kidney disease. The immediate effects of hypoglycemia can be severe including changes in cardiovascular and central nervous system function, cognitive impairment, increased risk for unintentional injury, coma, and death. Reducing the risk and impact of hypoglycemia would profoundly improve the quality-of-life for type 1 diabetes patients.

Normally, a drop in blood sugar triggers the body's warning system to release stress hormones, including adrenaline, resulting in symptoms such as shaking and sweating. The body also compensates with other "counter-regulation" defense measures including the release of glucagon, a hormone that elevates blood glucose. However, in type 1 diabetes, glucagon release does not occur and hypoglycemia warning signals are not often triggered due to an impaired adrenaline response. The individual does not recognize and therefore cannot correct for the low blood sugar—a syndrome known as hypoglycemia unawareness. Patients, especially children, are particularly vulnerable to hypoglycemia unawareness while they are asleep. Therefore "nocturnal hypoglycemia" is a primary concern and the source of many anxious nights for parents of type 1 diabetic patients who stay awake to check on the well-being of their children throughout each night. The autonomic nervous system that controls the activation of counter-regulation and warning-signal hormones that prevent hypoglycemia become progressively impaired in type 1 diabetes, in large part possibly because of brain alterations that occur during each hypoglycemic episode. This vicious cycle of recurrent hypoglycemia is referred to as hypoglycemia-associated autonomic failure, or HAAF.

The widespread introduction and use of reliable, accurate, and relatively "user-friendly" self-monitoring glucose devices and portable insulin pumps has transformed the management of type 1 diabetes in the past two decades. Yet, because current therapy still requires painful finger-sticks and injections, and in most cases still fails to achieve target glucose levels, it is critically important to develop non-invasive, continuous-monitoring and improved insulin delivery technologies. In addition, designing a "closed-loop" delivery system or "artificial pancreas"—made by combining the glucose sensor and insulin pump—is the next important step in achieving close glucose control, until islet or beta cell replacement therapy becomes a viable option for type 1 diabetes patients.

RECENT SCIENTIFIC ADVANCES

The phenomenon of hypoglycemia unawareness was originally described decades ago with the introduction of exogenous insulin; however, only recently have scientists begun to understand the complicated molecular mechanisms involved. This research, coupled with technological improvements in insulin therapy, is reducing the burden of hypoglycemia in type 1 diabetes.

Brain and Peripheral Metabolic Sensing: Maintenance of normal sugar balance (homeostasis) not only depends on the pancreas to release hormones in response to glucose levels, but it also requires the communication of signals from all over the body with the brain. In the past decade, significant advances have revealed where and how the brain measures the body's metabolic status. To measure glucose levels in the blood, cells with specialized molecular sensors—some of which are similar to those used by the pancreas—line vessels that lead to the liver and brain, as well as the gastrointestinal tract. These peripheral sensors are linked to groups of specialized glucose sensing nerve cells (neurons) which are localized within a distributed, interconnected network within the brain, including the hypothalamus, forebrain and hindbrain. To help the brain integrate different signals, some of these same brain neurons also respond to a variety of metabolic substrates (e.g., lactate, ketone bodies, fatty acids) and hormones (e.g., insulin, leptin, corticotropin releasing hormone), which are involved in the control of metabolism in the body. Identifying molecules and pathways for metabolic sensing may lead to targeted drug development to reduce hypoglycemia.

Hypoglycemic-Associated Autonomic Failure (HAAF): Why do most type 1 diabetes patients suffer from recurrent bouts of hypoglycemia? The reasons became clear with the discovery that treatment-induced hypoglycemia reduces defenses against subsequent hypoglycemic episodes. Researchers have long recognized that patients with type 1 diabetes do not secrete glucagon in response to hypoglycemia despite their ability to secrete glucagon under other circumstances. Recent findings suggest that a decrease in intraislet insulin is necessary for glucagon secretion, a response that does not occur in these patients. Identification and characterization of defects in the adrenaline warning-system and autonomic response that commonly appear as insulin therapy is intensified (HAAF) have contributed to the understanding of hypoglycemia unawareness and has laid the groundwork for future treatment.

Adaptive/Maladaptive Brain Responses: Therapies designed to protect the brain from injury due to hypoglycemia require a basic understanding of brain fuel usage and its adaptation to recurrent episodes of hypoglycemia. Recent progress has revealed how glucose and other fuels are transported into the brain despite a blood-brain barrier that blocks most molecules from entry. Surprisingly, new measurements show that glucose levels bathing the brain are only 25 percent of those in blood, which indicates that the glucose supply is very tenuous, particularly during hypoglycemia. Recent studies in rodents suggest that glucose transport into the brain may be increased by prior exposure to hypoglycemia and that brain glycogen ("starch") may also serve as a short-term fuel reserve to partially protect the brain from injury. Studies in patients suggest that the brain may more efficiently use other (non-glucose) fuels to meet its energy needs. Ironically, while these mechanisms do partially protect the brain from being damaged by impending hypoglycemia, they attenuate the ability of the individual to actually recognize and respond to hypoglycemia quickly (i.e., before dangerously low glucose levels impair brain

function). With complex factors modifying cognitive outcomes in patients, it is not yet fully clear how type 1 diabetes and insulin treatment alter sensitivity of the brain to hypoglycemia.

Development of Animal Models of Hypoglycemia: The neural systems that sense and respond to hypoglycemia are localized in brain areas and peripheral organs not readily accessible for study in human beings. For this reason, the development of animal models has been critical for understanding how the brain detects and responds to single and repeated bouts of hypoglycemia. These models mimic many of the same neural, hormonal, and behavioral deficits seen in humans, and are beginning to yield important new information about the body's adaptation to recurrent hypoglycemia.

Insulin Analogues and Glucose Sensing Technology: Use of intensive insulin therapy to achieve near-normal averages for long-term blood sugar control delays the development of vascular complications of diabetes, but at the cost of a three-fold increase in the risk of severe hypoglycemia. The frequency and potential consequences of severe hypoglycemia are much greater in children than in adults. The development and widespread use of new forms of insulin have advanced the ability to provide more physiological insulin replacement. The insulin analogues have, to some extent, reduced the incidence of hypoglycemia and, coupled with new technologies available for blood glucose measurements, have made it easier for patients to control their blood glucose. In addition, the recent introduction of continuous glucose monitoring systems to guide insulin replacement is potentially one of the most important recent advances in the treatment of type 1 diabetes, because it opens the pathway to development of an artificial endocrine pancreas.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

The dysfunctions that lead to hypoglycemia derive from the effects of diabetes and insulin therapy on the brain. Therefore, to prevent or reduce hypoglycemia, research must focus on how the brain measures glucose levels, how it adapts to hypoglycemic events, and how neural pathways are involved in hypoglycemia unawareness and autonomic failure. Furthermore, a major research objective is to develop clinical interventions that enable patients to better control their insulin levels.

Brain and Peripheral Metabolic Sensing

In order for the nervous system to recognize if blood glucose levels are too high or too low, specialized cells both within brain tissue and in other tissues such as the gastrointestinal tract and pancreas must be able to detect small changes in blood glucose. Metabolic sensing is a new and burgeoning area of basic research that is critical for the development of therapeutic interventions for the prevention and treatment of hypoglycemia. In addition to characterizing the specific cellular mechanisms involved in glucose sensing, scientists will need to understand how the neural responses to glucose levels are modulated by factors in the blood such as hormones and metabolites, and how sensing mechanisms are integrated and signals are relayed to different areas of the brain and peripheral tissues. Localization of these brain areas and neural networks in humans can be achieved through improvements in the resolution and sensitivity of imaging

technologies (see Goal VI).

Research Objective—Define the Mechanisms and Modulators of Metabolic Sensing:

- **Identify and elucidate the mechanisms involved in glucose sensing in the brain.**

Multiple mechanisms involved in sensing blood glucose concentrations are being discovered. The same enzyme (glucokinase) that pancreatic islets use to measure glucose may play a role in the brain. Recent evidence suggests that glucose sensors use chloride or ATP-sensitive potassium ion channels (molecules that increase or decrease the electric state of brain cells allowing charged ions to pass in response to binding a signaling molecule). The enzyme that muscle and other tissues use to sense fuel deficits (AMP-kinase) may also be used by the brain sensors.

Future research will need to clarify the specific molecular mechanisms used by glucose sensing cells, and in particular how diabetes and prior exposure to hypoglycemia may alter these mechanisms. Because glucose sensing mechanisms are also evident in peripheral tissues including the pancreas, liver, carotid body, and gastrointestinal tract, further characterization of glucose sensing tissues in the periphery may provide a model for understanding brain mechanisms. Identification of the mechanisms involved in glucose sensing and how they are altered by diabetes and its treatment will facilitate the development of pharmacological agents targeted for hypoglycemia prevention.

- **Determine the hormonal and metabolic modulators involved in glucose sensing.**

Insulin, the hormone that causes hypoglycemia, may also alter responsiveness of glucose-sensing mechanisms and ultimately, the responses of the brain to hypoglycemia. Circulating peripheral insulin gains access to the brain and elicits changes in release of neuropeptides and neurotransmitters. Similarly, other metabolic hormones, such as leptin, corticotropin-releasing hormone or fuels, such as short- and medium-chain fatty acids, or nitric oxide, have been suggested as modulators of glucose sensing mechanisms. Finally, glucose sensing might be modulated via changes in the number of glucose transporters, by molecules that recognize glucose outside the cell and bring it inside, or via altered regulation of cellular receptors—molecules on the surface of cells that take up hormones and neurotransmitters so that they can do their work in the body.

In addition to nerve cells, the brain contains other types of specialized cells that may play a critical role during hypoglycemia. Specifically glia, non-neuronal supporting cells within the brain, have the capacity to provide a source of energy to neurons during hypoglycemia by supplying both lactate (an energy-yielding breakdown product of sugar) and glycogen (a form of stored glucose arranged in long chains that can be broken down when needed). Future studies will need to determine if altered glucose sensing in diabetes or following hypoglycemia might be mediated by changes in glial function. In keeping with this possibility, glial-derived lactate has been reported to act as a signaling molecule in glucose sensing neurons.

Brain Alterations in Response to Hypoglycemia

The brain appears to respond to the stress imposed by insulin-induced hypoglycemia by using mechanisms that try to: (1) ensure a continued supply of energy, and (2) protect the brain from damage. Some of these responses may help sustain cognitive performance, while others may sacrifice higher function to preserve basic survival processes. Still others may actually impair defense responses to subsequent hypoglycemic events, such as those that occur during hypoglycemia-associated autonomic failure.

Research Objective—Elucidate Brain Alterations in Response to Hypoglycemia:

- **Determine alterations in brain metabolism and function induced by recurrent hypoglycemia.**

Varied scientific approaches are required to define how metabolism in the brain changes or adapts to reduce the insult and injury from recurrent episodes of hypoglycemia. A first step is to establish animal models of hypoglycemia in diabetes that replicate adaptive mechanisms of the brain. This resource will enable researchers to test therapeutic agents, isolate brain sections in culture, and apply newly developed gene array technology directly to brain tissue, thereby facilitating identification of new protective mechanisms. Animal models would also be useful for protocols not ethically possible in humans, such as examining if the level of glucose control in diabetes modifies brain metabolism responses to recurrent hypoglycemia.

The relative importance of the mechanisms involved in the brain's attempt to protect itself from injury must be established so that ultimately they may be targeted for therapeutic intervention. Potential mechanisms to investigate include: increased transport of glucose into the brain, increased storage of glucose as glycogen, or alternative fuel utilization (such as lactate or fatty acids) as occurs normally during starvation. Approaches to these questions can be tested clinically with new state-of-the-art brain imaging technologies, particularly Positron Emission Tomography (PET) and Magnetic Resonance Spectroscopy (MRS).

Of significant clinical concern is the effect of recurrent hypoglycemia on cognitive function. While it is known that cognitive function is impaired by acute hypoglycemia, the impact of intensive insulin therapy and "hypoglycemia unawareness" on cognition is poorly understood, in part because of the limitations of standard cognitive testing procedures. This issue can be addressed clinically with sensitive tools such as functional Magnetic Resonance Imaging (fMRI). This technology allows scientists to directly image the local activity of the working brains of patients while they are performing cognitive tests under conditions of hypoglycemia. Finally, insulin might itself alter cognition. Thus, studies need to determine the contribution of insulin *per se* to the changes in cognition seen during insulin-induced hypoglycemia.

- **Prevent hypoglycemia induced brain injury and promote protective adaptations.**

A major goal of uncovering both the adaptive and maladaptive mechanisms occurring in response to hypoglycemia is to develop therapies to reverse brain damage. Studies using state-of-the-art imaging technologies to assess brain function and metabolism could be performed in patients in whom hypoglycemic events are virtually eliminated, such as those patients treated with an artificial pancreas or an islet transplant. These patients would be

compared to type 1 diabetes patients with and without frequent episodes of hypoglycemia and hypoglycemia unawareness. Understanding of natural protective adaptations of the brain might lead to interventions to further promote protection from injury, as well as cognitive performance during hypoglycemia. For example, if the brain protects itself by increasing its use of alternative fuels, patients could take oral supplements to augment their supply of these fuels. If the brain burns glycogen during hypoglycemia, patients could take agents that promote glycogen storage.

- **Identify potential genes involved in individual susceptibility to hypoglycemia.**

Little is known about the potential influence of genetic risk factors in patients who suffer from frequent episodes of severe hypoglycemia. Angiotensin converting enzyme (ACE) may play a role, because high ACE activity and the presence of the D allele of the ACE gene is known to predict a high rate of severe hypoglycemia in type 1 diabetes. However, further genetic screening of highly-susceptible patients could help identify other genes and, potentially, prevention strategies.

Hypoglycemia-Associated Autonomic Failure (HAAF)

HAAF occurs as a result of episodes of hypoglycemia that commonly occur during insulin therapy. These episodes suppress and lower the level of glucose at which subsequent counter-regulatory responses occur, particularly the release of the hormone adrenaline. Adrenaline is the critical defense against hypoglycemia for type 1 diabetes patients because they already lack natural insulin and glucagon responses to hypoglycemia. Furthermore, suppression of adrenaline--as well as sympathetic nervous system responses--reduces the symptoms of low blood sugar, such as increased heart rate, sweating, and a desire to eat. It is these warning symptoms that alert patients to take corrective action.

HAAF can be reversed by as little as several weeks of scrupulous avoidance of hypoglycemia. Unfortunately, this is extremely difficult to accomplish in clinical practice without causing deteriorating glucose control—an undesirable consequence with respect to diabetes complications. Thus, it is of critical importance to define the currently unknown mechanisms responsible for HAAF so that new clinical strategies could be developed to prevent, correct, or compensate for HAAF, while at the same time, improve blood sugar control.

Research Objective—Develop New Strategies to Prevent or Reverse HAAF:

- **Elucidate the mechanisms of HAAF.**

Given the lack of understanding of the HAAF phenomenon, prevention will require experiments in animal model systems before rational therapies to enhance hormone defense systems in diabetes can be developed. Animal models afford the opportunity to determine at the molecular level whether hypoglycemia itself alters the function of glucose-sensing nerves, and, if so, how this occurs. For example, is it due to a change in nerve cell signaling, in metabolic function of the cells, in circulating or brain derived hormones, in brain neurotransmission, or a combination of some of these factors?

Furthermore, it is important to develop animal models of HAAF that not only replicate the pathophysiology of diabetes in humans, but are also consistent among laboratories. This

approach will facilitate the establishment of a central repository and/or resource listing for molecular probes, cell lines and transgenic animals for further HAAF research studies. A greater understanding of the putative molecular mediators leading to defective hormone responses in type 1 diabetes will provide the basis for testing unique therapies aimed at reversing HAAF. These ideas would initially be probed in animal models and eventually translated to clinical drug trials to test if the responses to acute hypoglycemia are improved and to see if they reduced hypoglycemic risk.

- **Identify the clinical consequences of HAAF.**

In humans, HAAF can be examined by applying newly improved imaging technologies, such as PET, fMRI, or MRS, to study the activation and integration of specific areas of brain that trigger hormonal defenses against hypoglycemia. Localization of region-specific activation patterns in humans will depend on continued improvements in the sensitivity of imaging techniques. These imaging studies should be performed in conjunction with functional monitoring of neuroendocrine, metabolic, and peripheral nervous system responses, as well as cognitive and behavioral responses. Integrative, multidisciplinary studies of this nature are required to investigate the broad pathological consequences of HAAF in humans. For example, recent studies demonstrate that the sympathetic nervous system's responses to hypoglycemia are reduced during sleep. Therefore, patients with type 1 diabetes are less likely to be awakened by hypoglycemia. This sleep-related HAAF, in the context of imperfect insulin replacement, probably explains the high frequency of nocturnal hypoglycemia in type 1 diabetes. Further studies are required to directly assess the impact of prior hypoglycemia at night on the counter-regulatory and neural responses during both sleep and exercise.

- **Develop and test therapies to restore counter-regulation.**

The complete loss of glucagon response to hypoglycemia develops in nearly all type 1 diabetes patients within a few years of diagnosis, independent of HAAF. Undoubtedly, if the glucagon response to hypoglycemia could be restored, the problem of hypoglycemia would be greatly minimized. Current data suggest that the inability of patients to suppress their intraislet insulin secretion may in part explain the absent glucagon response. Mechanistic studies characterizing the glucagon defect at the whole organ, cellular, and molecular level would therefore have great potential benefit for the development of agents to overcome the glucagon defect because the alpha cell works normally in type 1 diabetes patients at other times.

Clinical Interventions To Prevent or Reduce Hypoglycemia

Treatment of type 1 diabetes with exogenous insulin replacement will not be optimal until there is feedback control of insulin delivery, accomplished either by beta-cell replacement or by a mechanical, closed-loop insulin delivery system based on continuous glucose monitors (CGMs). Children and adolescents are particularly vulnerable to hypoglycemia. They are an ideal target population for closed-loop insulin delivery, because they are likely to receive the greatest benefit from it and are not appropriate candidates for islet transplants or other experimental approaches that currently involve lifelong immunosuppressive therapies.

The development of accurate and reliable continuous glucose monitors is the first step towards closed-loop insulin delivery. In comparison to the mature home glucose meter technology that has benefited from 25 years of development, CGM technology is still in its infancy and needs further refinement. As has been demonstrated by the Diabetes Research in Children Network (DirecNet), the devices that are currently approved by the FDA have major limitations.

Progress towards development of an artificial pancreas is likely to be a step-wise, iterative process. Several new and improved "real-time" CGM systems may be introduced, followed by the development of algorithms that allow for appropriate insulin delivery via continuous delivery systems.

***Research Objective—Develop New Approaches to Control Blood Glucose and Prevent Hypoglycemia*:**

- **Close the loop: develop the tools required for an artificial pancreas.**

Currently approved continuous glucose monitors are limited in their ability to reliably detect low blood glucose. Reliable identification of hypoglycemia in the home setting would minimize the risks associated with hypoglycemic events and permit the evaluation of factors, such as exercise and diet on the risk of hypoglycemia, especially during the night. Once reliable sensors are developed, it will be possible to conduct clinical trials of real-time glucose sensor monitoring with outcome measures, including reducing the risk of hypoglycemia, lowering HbA1c levels, and enhancing counter-regulatory hormone responses using insulin pumps or basal/bolus injection regimens. As part of these investigations, guidelines could be developed on how to use glucose sensor data to optimize glucose control and minimize the risk of severe hypoglycemia.

The development of computerized algorithms that automatically vary insulin delivery rates based on glucose sensor data is essential to providing adequate control of postprandial hyperglycemia and eliminating the risk of hypoglycemia. Further studies would test how robust such systems are over time and under a variety of real-life conditions. Once reliable sensors are developed and appropriate algorithms are established, their integration into a closed loop system would be feasible. The development of closed-loop insulin delivery would initially start with external, minimally invasive, subcutaneous, short-term sensors and external pumps and would lead ultimately to fully implantable, long-term systems. This progress will require cooperation between the NIH, industry, and foundations. Moreover, it will require enhanced collaborations between diabetes specialists, islet physiologists, bioengineers, and the computational biology/informatics community.

- **Evaluate behavioral approaches to preventing nocturnal hypoglycemia.**

Nocturnal hypoglycemia remains a significant clinical problem and source of concern for parents of children with type 1 diabetes. A practical aid to them in helping their children maintain normal blood sugar levels would be a systematic evaluation of the ways that the dietary composition of evening meals and snacks can be varied to reduce the incidence of nocturnal hypoglycemic events and subsequent morning glucose values.

Table 4. Key Research Objectives for Prevention and Reduction of Hypoglycemia

- Define the Mechanisms and Modulators of Metabolic Sensing
- Elucidate Brain Alterations in Response to Hypoglycemia
- Develop New Strategies to Prevent or Reverse Hypoglycemic-Associated Autonomic Failure (HAAF)
- Develop New Approaches to Control Blood Glucose and Prevent Hypoglycemia

GOAL V: PREVENT OR REDUCE THE COMPLICATIONS OF TYPE 1 DIABETES

Why This Goal Is Important to People

The constant companion of patients and their loved ones is fear of the complications of diabetes. The newly diagnosed patient asks: "If my average blood sugar level is good, how dangerous is the occasional high? Will I lose my vision? Will I need an amputation? Can I be tested to find out my risk of developing complications?" Patients who already have one or more of the complications ask: "What, if anything, can I do to limit the damage that diabetes is causing to my body?"

Type 1 diabetes ravages nearly every part of the body: the heart, eyes, kidneys, nerves, lower limbs, mouth, and digestive and urologic systems. Many people with type 1 diabetes are coping with its devastating complications, such as kidney failure, blindness, nerve pain, and heart disease. Others with diabetes—including children—fear that this will be their future.

Destructive Complications of Type 1 Diabetes

For those with type 1 diabetes, life expectancy may be shortened by about 15 years (4), with heart attacks and strokes the primary cause of premature death (5). Cardiovascular disease strikes people with type 1 diabetes at greater rates, and treatment is particularly difficult, because the effects of type 1 diabetes reduce the success of established cardiovascular therapies. The daily lives of many people with type 1 diabetes are made harder as a result of vision loss from diabetes. Patients with diabetes also face increased risk of irreversible kidney disease (end-stage renal disease), leading to the requirement for either dialysis for the remainder of their lives, or a kidney transplant.

Amputation of the lower extremities is too frequently the end result of non-healing foot ulcers. Patients with diabetes can lose sensation in the legs and feet because of diabetic nerve damage. The consequent inability to perceive pain allows the silent development of foot ulcers, which then fail to heal because of insufficient blood flow and other factors secondary to diabetes.

Among other complications of this disease, diabetic nerve damage can cause erectile dysfunction, urinary incontinence, and nocturnal diarrhea. Women with type 1 diabetes face additional health risks during pregnancy, and diabetes is associated with increased risk of birth defects in their children. Gum disease and other oral health problems are also complications of diabetes.

Because the prevention or cure of type 1 diabetes is not yet possible, intensified research toward preventing and treating the complications of diabetes is critically important. This research would have overwhelming benefits for people with type 1 diabetes. It would have additional health and

economic benefits by improving the lives of the millions of Americans with type 2 diabetes, who suffer many of the same complications.

Identifying "Targets" for New Diagnostics and Therapies—Learning How Diabetes Leads to Complications

Insulin Deficiency: The path from the onset of type 1 diabetes to the development of severe complications begins with insulin deficiency. Researchers are vigorously working to devise ways of replenishing the insulin-producing cells that are destroyed by type 1 diabetes, as described in Goal III. However, because of the extraordinary complexity of replacing or regenerating these cells, scientists are also accelerating research to target other points along the path from diabetes to its complications.

Elevated Blood Glucose: One immediate result of insulin deficiency is high blood glucose levels, termed "hyperglycemia," a hallmark of type 1 diabetes. Scientists have demonstrated that intensive control of blood glucose levels can have long-lasting effects toward reducing onset of complications. Such intensive glucose control was achieved in type 1 diabetes patients in the landmark Diabetes Control and Complications Trial (DCCT) through more frequent monitoring of blood glucose levels than was conventional, along with more frequent insulin injections or use of a pump. In an ongoing follow-up effort, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, researchers are continuing to evaluate the health of the DCCT participants, including those who had been assigned to intensive treatment, as well as those participants who had been in a conventional treatment group. After the DCCT ended, those in the conventional group improved their glucose control but the individuals who had been in the intensive treatment group were unable to maintain such strict control of their blood glucose levels. Surprisingly and provocatively, however, the effects of their finite time of intensive control have persisted for years. That is, compared to the DCCT participants from the conventional treatment group, those who were in the original intensive treatment group continue to have lower incidence of complications—eye and kidney disease, heart attacks and stroke—even 10 years later, despite similar levels of glucose during this period. In contrast, the effects of higher glucose exposure for a finite time in the DCCT participants from the conventional group have also persisted, causing increased complication rates despite long-term improvement of hyperglycemia. The phenomenon of long-lasting effects of a period of intensive or non-intensive glucose control has been termed "metabolic memory." The discovery of the molecular and cellular basis of metabolic memory is urgently needed so that therapies can be designed to mimic or induce the body's protective "memory" of good control of blood glucose levels and to counteract the harmful "memory" of higher glucose levels.

Mechanisms By Which Hyperglycemia Causes Damage: Another strategy for blocking the path from type 1 diabetes to its complications is to impede the processes by which high glucose levels cause cell and tissue damage. Pursuing this approach, scientists have recently suggested that a variety of deleterious molecular effects of diabetes may all arise from a single, hyperglycemia-induced process: the overproduction of a molecule called superoxide. Several novel agents based on this pathway have shown promise in pre-clinical experiments, and will be evaluated in clinical studies in type 1 diabetes patients.

Additional Targets for Therapeutics Development--Blood Vessel Damage and Repair Pathways, Inflammation, and Abnormal Lipid Processing: Other advances in understanding the molecular events leading to diabetic complications will spur the design of potential new therapies. For example, a key aspect of diabetic complications is the underlying damage to blood vessels throughout the body. Scientists recently found that diabetes not only leads to blood vessel damage, but also may impair the regeneration of healthy new blood vessels. Novel drug or cell-based therapies to induce new blood vessel growth (angiogenesis) in type 1 diabetes patients may help promote wound healing and assist with repair of diabetes-induced damage to the heart and nerves. In contrast, excessive angiogenesis contributes to diabetic eye disease; limiting new blood vessel growth in the eye may be beneficial. Cancer researchers have developed effective new cancer drugs targeted at angiogenesis. This strategy is now being utilized to identify therapeutic agents to prevent or treat diabetes complications. Other researchers are focusing on the abnormal metabolism of fats in type 1 diabetes, including the toxic accumulation of fatty acid molecules in heart cells. Insights into yet another detrimental consequence of diabetes, activation of molecular pathways related to inflammation, are also providing opportunities for intervention.

Advancing Technologies for Developing Therapies

In conjunction with searching for therapeutic targets by mining the expanding knowledge base of molecular processes that lead to diabetic complications, researchers have also embarked on a new strategy to accelerate the discovery of compounds that could act as drugs. A component of the NIH Roadmap for Medical Research involves automated synthesis of large numbers of chemical compounds, and large-scale, rapid screening of these compounds for therapeutic potential. A large set of such compounds is termed a library. "High-throughput screening" refers to the testing of large numbers (i.e., a library) of compounds to see whether any show promise as potential drugs. To improve high-throughput screening techniques for drugs for diabetic complications, it will be important to employ biological assays that not only can be performed rapidly in the lab, but that also reflect what is happening in the body as complications develop. Drawing upon the emerging understanding of the molecular processes leading to diabetic complications, scientists may soon be able to design new biologic assays that mimic these processes in the laboratory, for use in high-throughput screens.

Compounds identified as potentially useful by a high-throughput screen for diabetic complications can then be more intensively investigated to choose the most promising candidates for clinical trials. A crucial step in this selection process is testing compounds in animal models for diabetic complications. Suitable animal models that mimic the human condition are critical for the success of drug development. Efforts are underway to create such models by drawing upon new understanding of the molecular pathways underlying development of complications.

Facilitating Clinical Trials and Clinical Care: "Biomarkers" as Early Molecular Signs of Diabetic Complications

The multi-organ damage caused by type 1 diabetes progresses silently for many years before signs or symptoms become apparent. Even more years may elapse before complications reach the severity of a heart attack, kidney failure, or other devastating event. Very early detection of

the development of complications could permit early, successful intervention and reduced suffering for type 1 diabetes patients. Thus, a critical area for research is the discovery and evaluation of "biomarkers" for early detection of damage to cells and tissues. For example, the abnormal excretion of small amounts of protein in urine is currently used as a biomarker for early diabetic kidney disease, before organ deterioration to kidney failure. Early intervention based on this biomarker has been credited with the recent slowing in rates of kidney failure in the U.S. The molecular processes along the path from insulin deficiency to development of complications, discussed earlier, present rich opportunities for the discovery of new biomarkers. Scientists are also exploring non-invasive imaging techniques as a means of detecting disease progression.

Biomarkers research will also facilitate and expedite clinical trials. The development of therapeutics for diabetic complications is severely constrained because of the relatively slow progression rate of these complications. Clinical trials must therefore extend for long durations in order for researchers to detect the effect of a candidate drug. "Surrogate endpoints" are biomarkers that are strongly associated with and predictive of disease outcomes. Valid surrogate endpoints can be used in shorter clinical trials to choose the most promising drug and trial conditions prior to longer clinical trials assessing the definitive clinical endpoint. However, the standards for acceptance of new biomarkers and surrogate endpoints are extremely high, adding to the challenges of this area of research.

Predicting Risk of Complications

The occurrence and progression of diabetic complications varies markedly among patients. Many factors contribute to the risk for complications, including genetic variation. Several research consortia are conducting studies to identify genetic factors that confer susceptibility or resistance to diabetic complications, including the Genetics of Kidneys in Diabetes Study (GoKinD) and the Family Investigation of Nephropathy and Diabetes (FIND), as well as a component of the EDIC study. Results of this research may help inform patient care, discover new mechanisms of disease progression, and aid evaluation of potential new therapeutics.

By elucidating the cellular and molecular processes by which type 1 diabetes progresses to complications, and by propelling research on detection methods and advanced techniques for drug development, scientists will greatly improve the lives of people with this disease. These efforts will reduce the heightened risk for heart disease, kidney failure, blindness, and other debilitating, costly, and deadly complications of diabetes. A great hope of this research is that children and adults with diabetes today will have a much brighter future.

GOAL V: PREVENT OR REDUCE THE COMPLICATIONS OF TYPE 1 DIABETES

INTRODUCTION AND BACKGROUND

For a child at the beginning of the 20th century, the diagnosis of type 1 diabetes was equivalent to a death sentence. The discovery of insulin commuted that death sentence, but it soon became apparent that insulin treatment allowed many of these children to live just long enough to develop diabetes-induced blindness, kidney failure, and coronary disease. At the beginning of the 21st century, type 1 diabetes still has a profound effect on many people's lives, because these long-term health complications that affect nearly every organ system in the body still occur with alarming frequency.

Patients with type 1 or type 2 diabetes face the possibility of similar complications. For patients and their families, the grim statistics about complications are far too familiar.

- Life expectancy may be shortened by about 15 years (4) with premature deaths due primarily to heart attacks and strokes (5). Rates of cardiovascular disease are increased up to 10-fold compared to those in the age-matched general population (2, 3). In addition, diabetes impairs repair pathways necessary for the success of established cardiovascular therapies such as coronary angioplasty and bypass grafting, and lower extremity revascularization. This causes a significantly lower success rate in treating vascular diseases in diabetes patients.

- In addition to injuring the large vessels of the heart and brain, diabetes also damages the small blood vessels of the body, called the microvasculature. Diabetes (type 1 and type 2) is now the leading cause of new blindness in people 20 to 74 years of age (5). In those with type 1 diabetes, retinal damage (retinopathy) is detectable in virtually all patients after twenty years as a consequence of damage to the microvessels of the retina.

- Diabetes also leads to protein in the urine (microalbuminuria) and eventually irreversible kidney disease (nephropathy) which progresses to end-stage renal disease, requiring dialysis or kidney transplantation. Importantly, only about one third of type 1 diabetes patients appear to be susceptible to this devastating complication (19). The factors that determine susceptibility to nephropathy for this group of patients (or protect others) are still unknown.

- More than 60 percent of diabetes patients are affected by painful nerve damage and loss of sensation (neuropathy), particularly in the legs and feet (5). Foot ulcers often arise because of the inability to perceive pain and then fail to heal because of insufficient blood flow and other factors secondary to diabetes. Amputation of the lower extremities is too frequently the end result of non-healing ulcers. Diabetic nerve damage also contributes to erectile dysfunction, urinary incontinence, and nocturnal diarrhea.

- Other complications of type 1 diabetes include increased rates of birth defects in children of mothers with diabetes and severe periodontal disease.

Preventive strategies are beginning to reduce the incidence of diabetes complications. Nonetheless, tight control of blood glucose levels is difficult to achieve because of the risks of dangerously low blood sugar (hypoglycemia) and the need for unrelenting vigilance about dietary intake, the monitoring of blood glucose levels, and the administration of insulin. Good control of blood pressure and blood lipid levels--as well as careful monitoring for retinal damage, albumin in the urine, sores on the feet, and other signs and symptoms of diabetes--are all measures for preventing or mitigating complications of diabetes. Although blockade of the renin-angiotensin system is well established to slow the progression of diabetic nephropathy, it does not always prevent the development of renal failure. The treatment options for other complications are even less satisfactory. In particular, symptomatic treatment for nerve pain is poor. While laser photocoagulation has been an extremely important advance for treating diabetic retinopathy, vision loss from this complication is not always preventable, even with the best interventions.

In type 1 diabetes, complications are due to metabolic derangements caused by loss of insulin resulting from the autoimmune destruction of insulin-producing cells. The best understood metabolic defect causing diabetic complications is elevated blood glucose (hyperglycemia) that can modify the extracellular environment and, in some cell types, directly lead to excess glucose inside the cell. Intracellular hyperglycemia initiates a cascade of changes in cell metabolism that includes increased production of reactive oxygen species, increased sugar-modified proteins, and activation of a number of signaling pathways. In addition to causing hyperglycemia, insulin deficiency can also contribute to end-organ damage in type 1 diabetes through alterations in the metabolism of lipids and lipoproteins. In the endothelial cells that line the blood vessels in the eye, kidney, nerve, and heart, these alterations lead to increased leakiness of blood vessels, decreased vascular density, and inadequate delivery of blood due to cell loss, and altered expression of cell-surface proteins that initiate and perpetuate a damaging inflammatory response. Tissue responses to these cellular changes in the blood vessels are specific for each tissue and organ, with genetic variation playing an important role in determining the nature and extent of these responses for each individual.

Advancing scientific knowledge of diabetic complications to improve clinical care is a multi-dimensional challenge. Meeting this challenge would have overwhelming benefits for people with type 1 diabetes. New discoveries about diabetic complications may also improve the lives of millions of Americans with type 2 diabetes, who also suffer from the same devastating complications.

RECENT SCIENTIFIC ADVANCES

Significant progress in understanding diabetic complications has occurred in the past decade. These discoveries are leading to the development of effective therapies to prevent and treat the cell, tissue and organ damage caused by diabetes.

Progress in Reducing Diabetic Nephropathy: Recent reports indicate that prevention efforts are beginning to have dramatic affects on the rates of diabetic nephropathy in type 1 patients. This devastating complication of diabetes has historically been seen in as many as one-third of diabetic individuals after 20 or 30 years of disease (19). In the most recent population-based

study from Finland, however, only 7.8 percent of type 1 diabetes patients have renal failure after 30 years of diabetes (20). Declines in the incidence of end-stage renal disease due to diabetes are being noted for the U.S. population as well, in reports from the United States Renal Data System. These gains are most noteworthy in diabetic patients under age 30 (most of whom have type 1 diabetes) and are restricted to Caucasians and not observed in African-Americans. The rate of end-stage renal disease in Caucasians under 30 with diabetes is almost half the rate seen in the late 1980s and early 1990s (9). Since that time, several clinical strategies have been proven to significantly reduce the progression of diabetic nephropathy. These include angiotensin-converting enzyme inhibitors (ACE-inhibitors) and angiotensin receptor blockers (ARBs), which lower protein in the urine and are thought to directly prevent injury to the kidneys' blood vessels; and careful control of blood glucose and blood pressure. Credit for the recent gains likely goes to implementation in clinical practice of these strategies to prevent disease, including better glycemic control, hypertension management, and use of ACE inhibitors and ARBs. Research to build on this success and extend it to all populations in the U.S. is a high priority for the NIH.

Role of Reactive Oxygen Species (ROS) in Complications Pathogenesis: Over the past 35 years, several molecular mechanisms have been implicated in glucose-mediated vascular damage. Each of these mechanisms has been studied independently of the others, and there has been no apparent common element linking them. Recent discoveries have made clear that all of these seemingly unrelated mechanisms may arise from a single, hyperglycemia-induced process: the overproduction of the reactive free radical molecule, superoxide. It now appears that the energy-generating cellular organelles called mitochondria are required for the initiation of hyperglycemia-induced superoxide production, which can, in turn, activate a number of other superoxide production pathways that may amplify the original damaging effect of hyperglycemia. Increased free fatty acid oxidation in mitochondria produces superoxide as well. In diabetic mice genetically engineered to produce high levels of an enzyme that degrades superoxide (called "superoxide dismutase"), diabetes fails to activate any of the classic hyperglycemia-induced damaging pathways and these mice do not develop diabetic kidney disease. This advance not only serves as a focus for future research in diabetic complications, but also indicates that a single therapy directed at overproduction of reactive oxygen species might ameliorate the multi-organ effects of diabetes. Several novel pharmacologic approaches based on this unifying mechanism have already prevented diabetic eye, kidney, and nerve pathology in rodent models of diabetes.

Therapeutic Approaches Based on a Soluble Form of the Receptor for Advanced Glycation Endproducts (RAGE): Although elevated levels of low-density lipoproteins (LDL cholesterol) appear to be required for atherosclerosis, the pathologic result for any given level of LDL depends on a wide variety of other factors, many of which are proinflammatory molecules secreted by a variety of tissues and cell types. Recently, it has been established that nearly all people with type 1 diabetes have accelerated atherosclerosis and coronary artery disease, and that this is associated with increased local tissue levels and blood levels of proinflammatory molecules. A major advance in this field is the identification of RAGE, a component of the innate immune system originally identified by its ability to bind sugar-modified proteins known as Advanced Glycation Endproducts (AGE). RAGE is found in many tissues, including those prone to diabetic complications. The binding of AGE to RAGE activates signaling pathways that lead to generation of reactive oxygen species. In addition, a soluble form of RAGE binds a

number of proinflammatory factors such as S100 calgranulins and high mobility group box 1 (HMGB1), preventing their interaction with cellular receptors. Treatment with this soluble form of RAGE prevents accelerated atherosclerosis in several models of experimental diabetes. In addition, soluble RAGE blocks kidney disease in diabetic mice. Because soluble proteins are not ideal pharmacologic agents in all settings, especially for those that require chronic administration over many years, additional research focused on the development of orally-available antagonists (drugs that block the receptor), and modification of both the inflammatory peptides and the receptor, should lead to the development of important new therapies to retard atherosclerosis for people with diabetes.

Diabetic Heart Damage from Accumulation of Fatty Acids: A significant advance in understanding diabetic heart disease is the discovery that derangements in cardiac fuel utilization are, at least in part, responsible for myocardial disease caused by diabetes. Both cardiac and skeletal muscle cells (myocytes) have high energy requirements. Cells normally use both glucose and fatty acids as energy sources. In diabetic heart muscle, insufficient insulin action reduces transport of glucose into the cells. To compensate, these cells switch to fatty acids as their primary energy source with chronic activation of the nuclear receptor PPAR-alpha mediating this switch. The increased flux of fatty acids into heart muscle cells overwhelms the mitochondria's ability to burn this fuel. The result is intracellular accumulation of a variety of fatty acid metabolites, whose deleterious effects on cells have been termed "lipotoxicity." Accumulation of fatty acid metabolites leads to death of cardiac myocytes in hearts of diabetic animals, and makes these cells especially sensitive to further damage from hypoxia associated with angina or a heart attack. These findings may lead to therapies that increase glucose uptake and utilization in heart muscle, and thereby reduce the damage caused by accumulation of fatty acids. These results could also lead to the development of diagnostic imaging methods to detect deleterious changes in cardiac cell metabolism before they lead to heart disease.

Insulin Resistance--an Independent Cardiovascular Risk Factor in Type 1 Diabetes: In some regards, the classic distinction between type 1 and type 2 diabetes in beginning to break down. Type 1 diabetes is a disease in which there is a loss of insulin production due to autoimmune destruction of insulin-producing cells. In type 2 diabetes, loss of effective insulin action is due to a combination of defects both in normal insulin action (insulin resistance) and in the ability of pancreatic beta cells to overcome this insulin resistance by secreting enough additional insulin. Over the past ten years, evidence has mounted to show that, in type 1 diabetes, hyperglycemia itself eventually causes secondary insulin resistance in nearly all patients. Because insulin resistance appears to operate as a significant factor in the development of cardiovascular disease, independent of other mechanisms such as lipid abnormalities and high blood pressure, the discovery that hyperglycemia causes insulin resistance in type 1 diabetes links accelerated atherosclerosis in type 2 patients with accelerated atherosclerosis in type 1 diabetes. Moreover, this discovery suggests novel pharmacologic approaches for reducing cardiovascular disease in type 1 diabetes patients based on direct reduction of insulin resistance.

Impaired Blood Vessel Formation from Bone Marrow Progenitor Cells in Diabetes: Diabetic complications result not only from damage to cells and tissues, but also from the inadequacy of the repair process. During the acute response to injury, new blood vessel growth rescues "stunned" areas of the heart or central nervous system, reducing morbidity and mortality.

With chronic low perfusion, the development of collateral vessels reduces the size and severity of a subsequent infarction. Circulating progenitor cells from the bone marrow promote the regeneration of blood vessels by acting in concert with the cells and extracellular matrix at the site of injury. A major advance is the observation that these endothelial progenitor cells are depleted and dysfunctional in diabetes, and that injection of normal progenitor cells can improve blood supply to the tissues and nerve function in experimental diabetes. Research focused on the diabetes-induced impairment of this process could lead to novel drug- and cell-based therapies for people with diabetes to restore compensatory vessel formation in cardiovascular disease, stroke, peripheral vascular disease, and wound healing. In the diabetic retina, however, overly exuberant vascular repair processes can result in excessive proliferation of small vessels. Molecular pathways responsible for the new vessel growth have been identified, and this work suggests new molecular targets for drugs that could protect the retina.

Sustained Effect of Glycemic Control on Complications Susceptibility--"Metabolic Memory": In 1993, the results of the landmark Diabetes Control and Complications Trial (DCCT) showed that, in people with short-duration type 1 diabetes, intensive glycemic control dramatically reduced the occurrence and severity of diabetic microvascular complications. After the announcement of the DCCT results, many patients who had been in the standard therapy group adopted more intensive therapeutic regimens, and their level of glycemic control improved, as measured by the hemoglobin A1c (HbA1c) test. At the same time, the mean level of HbA1c worsened for patients who had been in the intensive therapy group. The post-DCCT HbA1c values for both groups have become nearly identical during the approximate 10 years of follow-up in the on-going Epidemiology of Diabetes Interventions and Complications (EDIC) study.

Surprisingly and provocatively, however, the effects of a 6.5 year difference in HbA1c during the DCCT on the incidence of retinopathy and nephropathy have persisted, and have even become greater over the subsequent decade of follow-up. People in the standard therapy group continued to have a higher incidence of complications even with an improvement in glycemic control during the EDIC. In contrast, people in the intensive therapy group continued to have a lower incidence of complications, even with a worsening of glycemic control during EDIC. In addition, early intensive glycemic therapy was recently shown to markedly reduce later development of atherosclerotic changes, heart attacks, and strokes.

The phenomenon that glycemic control could have long-lasting effects is called metabolic memory and elicits a number of questions. How can a finite period of good or bad glycemic control have such long-lasting effects? Is there a point in the development of complications in which the progression becomes relatively independent of glycemic control? The discovery of the molecular and cellular basis of metabolic memory is urgently needed so that solutions can be designed to mimic or induce the protective "memory" of good glycemic control, and to inhibit or reverse the sensitizing "memory" of poor glycemic control.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

The prevention and reduction of complications will be greatly facilitated by the discovery and development of therapeutics that will prevent or reverse the cellular and tissue injury induced by

type 1 diabetes and hyperglycemia. Each research objective in this chapter addresses a critical area necessary to achieve this overarching goal. An understanding of the molecular mechanisms and genetic risk factors underlying diabetic complications may lead to the identification of new molecular targets for drug development. Application of the latest advances in drug development technology to diabetic complications has the potential to greatly decrease drug development time and improve prospects for clinical success. To test promising drug candidates, animal models are needed that more completely mimic the human pathology of diabetic complications. The discovery of biomarkers and surrogate end-points for the early manifestations of diabetic complications may allow targeted therapies and potentially shorten the duration of clinical testing, thus removing a significant barrier to achieving clinically useful therapeutics for diabetic complications.

Molecular Mechanisms of Common Pathways in Diabetic Complications

The understanding of the mechanisms underlying diabetic complications has greatly expanded in recent years as scientists have identified several implicated molecules. Recent technological advances present an important opportunity to fully characterize the disease pathways that cause retinopathy, nephropathy, neuropathy, cardiomyopathy, accelerated atherosclerosis, and other diabetic complications. Basic science discoveries--such as the recent discovery of microRNAs (see Goal VI), which may regulate expression of one-third of all genes--will help researchers studying complications to identify completely new and unpredicted therapeutic targets and clinically useful biomarkers.

Research Objective—Identify Molecular Pathways of Hyperglycemia Damage:

- **Discover the factors controlling hyperglycemia-induced reactive oxygen species (ROS) formation and adaptive and maladaptive cellular responses to increased ROS.**
- **Identify the molecular events controlling RAGE expression and endogenous soluble RAGE production.**
- **Discover the mechanisms by which hyperglycemia impairs bone marrow progenitor cell function, especially vascular cell progenitors needed to repair wounds and revascularize ischemic heart muscle, peripheral nerves, and lower limbs.**
- **Identify the mechanisms of vascular proliferation in diabetic retinopathy.**

The identification of the cellular and molecular pathways involved in diabetic complications provides a strong foundation for research on key regulatory steps in these pathways that should lead to exciting and clinically relevant discoveries. These breakthroughs in basic research on cellular pathways promote interdisciplinary research with investigators in other basic science and disease-based fields. The goals listed above on oxidative stress, inflammation (RAGE), and angiogenesis are central not only to diabetic complications, but are also involved in numerous other diseases such as cancer and atherosclerosis. Research in this area will benefit from and contribute to the much broader biomedical research endeavor.

Research Objective—Clarify Mechanisms Linking Fuel Utilization and Heart Disease:

- **Characterize the factors controlling increased fatty acid accumulation and oxidation in the development of diabetic cardiomyopathy and accelerated atherosclerosis, and the mechanisms by which this cellular lipotoxicity induce cell damage.**

The heart has extraordinarily high energy requirements related to its function as a pump throughout life. The energy demands of the heart are met through a high capacity mitochondrial system well-suited to oxidize fatty acids and glucose to generate energy. In the insulin-deficient state, this high capacity mitochondrial system is pushed to the limit through increased reliance on fatty acid oxidation as the energy source. Whereas the increase in cellular fatty acid utilization in the insulin-deficient state is initially an adaptive response, evidence is emerging that this increased fatty acid import and oxidative flux lead to deleterious consequences relevant to the pathogenesis of myocardial and vascular disease. Early studies in this area have identified a number of potential mechanisms linking increased fat utilization to cellular toxicity including the accumulation of lipid products that could trigger signaling events leading to apoptosis (cell suicide); generation of reactive oxygen species via increased oxidative flux through mitochondria and peroxisomes; and secondary damage to mitochondria leading to bioenergetic abnormalities. Future studies related to each of these potential mechanisms will be important for enhancing understanding of the pathogenesis of cardiovascular toxicity in type 1 diabetes. Moreover, identification of relevant cellular events involved in this response could pave the way for identification of new therapeutic targets and biomarkers.

Research Objective—Understand the Systems Biology of Diabetic Complications:

- **Apply a systems biology approach to research on diabetic complications.**

The pathogenesis of diabetic complications encompasses much more than the cellular responses to the metabolic defects of diabetes. Each known diabetes-induced abnormality within a cell can be thought of as connected in a circuit-like arrangement with other intracellular molecules. Similarly, the pathology in one cell type is also connected in a circuit-like arrangement with other cell types in a specific tissue, and each tissue type is likewise connected to other tissues and organ systems, with changes in one nodal component influencing many other points in the network. The emerging field of systems biology will have a major impact on progress in this area. Systems biology is a powerful, mathematically-based discipline that seeks to analyze the many simultaneously occurring changes in intracellular, intercellular, and inter-organ contexts as complex, interconnected circuits that have nodal control points, much like the electronic circuits on a microchip. The use of a systems biologic approach can lead to models of *in vitro* and *ex vivo* systems, both useful for identifying mechanisms of injury and testing targets for therapy.

Metabolic Memory

The phenomenon of hyperglycemic memory presents a paradox: Patients in the DCCT with long-term exposure to a higher level of hyperglycemia remained more susceptible to complications even with subsequent lower levels of hyperglycemia. In contrast, lower levels of hyperglycemia made patients more resistant to damage from subsequent higher levels. How can a finite period of different degrees of hyperglycemia result in different susceptibilities to complications? The discovery of the molecular and cellular basis of both types of metabolic memory is urgently needed so that solutions can be designed to prevent or reverse the damaging "memory" of high hyperglycemia, and to mimic or induce the protective "memory" of lower levels of hyperglycemia. Unlike the pathogenesis of diabetic complications, the molecular mechanisms underlying metabolic memory are virtually unexplored.

Research Objectives—Discover the Molecular Mechanisms of Metabolic Memory:

- **Study epigenetic factors involved in metabolic memory**.

The Director of the National Human Genome Research Institute recently noted that there is an emerging recognition that scientists must move beyond their long-standing focus on the inherited spelling of people's DNA code and the occasional mutation or outright "misspelling." He further noted that epigenetic changes do not alter genetic spellings but may account for as many cases of cancer and other diseases as full-blown gene mutations.

Human cells have tens of thousands of genes, each with its own job, such as producing energy or overseeing cell division. But only certain genes are active at any given time or in any cell type, while the rest are appropriately dormant—a grand orchestration that adds up to a smooth-running life. This orchestration is determined by environmentally-induced changes in molecules that coat the DNA. It has long been known that even identical twins have minor physical variations and differences in characteristics such as susceptibility to disease. Recently, two dominant epigenetic changes were studied in identical twins: in DNA methylation, enzymes inside a cell attach a minuscule molecular decoration to a gene, deactivating that gene; in histone acetylation, a dormant gene is made active again. These altered genetic settings can last a lifetime, and could be important for diabetic complications if hyperglycemia can lead to these permanent genetic alternations. For example, a period of hyperglycemia could irreversibly turn off a gene that protects against diabetic complications. The ability of hyperglycemia to elicit epigenetic changes may be associated with different stages of development, and therefore, could lead to treatment strategies that would promote intensive therapies during critical windows of development.

- **Investigate the role of mitochondria in metabolic memory.**

Much progress has been made in understanding the complex biology of mitochondria, the major source of hyperglycemia-induced reactive oxygen species (ROS). Scientists now recognize that mitochondria are not all the same, but rather have important functional differences. Furthermore, mitochondria are not static structures in the cell. Rather, they continuously fuse to form larger organelles, or pull apart, to form smaller organelles. The processes underlying these changes are beginning to be understood, but aberrations induced by diabetes and different degrees of hyperglycemia are important new areas that will likely yield important answers relevant to hyperglycemic memory.

- **Understand the regulation of the antioxidant response element.**

Perhaps not surprisingly, cells have their own protective antioxidant machinery. In a nematode model organism, cells responded to oxidative stress by activating a previously sequestered transcription factor (called Nrf2 in humans) that controls the expression of a diverse set of genes involved in decreasing ROS in the cell. An important research focus is the identification and regulation of proteins in this pathway, including proteins that bind to a special promoter element called the antioxidant response element (ARE) after activation by ROS. Such research will be critical to advance understanding of hyperglycemic memory.

Genetic Factors

As with all complex diseases, the occurrence and progression of diabetic complications varies markedly among patients. Some patients have type 1 diabetes for over 50 years with minimal complications, while others manifest severe disease or death within 15 years after diagnosis. The control of blood glucose, as well as blood pressure and blood lipid profiles, are important factors in predicting the risk of complications, but they only partially explain the risk of complications for an individual patient. Therefore, genetic factors have been investigated for their influence on the risk of developing complications. An understanding of the genes involved in the susceptibility to or protection from diabetic complications can also lead to both a better understanding of the pathophysiologic mechanisms, as well as new biomarkers and molecular targets for drug development.

Research Objective—Identify Genetic Factors of Susceptibility and Resistance to Diabetic Complications:

- **Determine the genes that increase susceptibility to diabetic complications.**

The DCCT and other independent studies of type 1 diabetes patients and their close relatives have shown that the incidence of nephropathy (and to a lesser extent retinopathy and neuropathy) in one sibling increases the risk that other siblings will develop the same complication. These studies provide evidence for a genetic component to the risk of developing complications. Possible candidate susceptibility genes have been selected based on the fact that they encode proteins thought to play a role in several known mechanisms relevant to diabetic complications. Using this candidate gene approach, researchers have made numerous associations between various genetic polymorphisms and the risk of various diabetic complications.

Current strategies to meet this objective involve three ongoing consortia that are addressing the genetic factors that predispose patients with diabetes to or protect them from developing complications: Genetics of Kidneys in Diabetes Study (GoKinD), the Family Investigation of Nephropathy and Diabetes (FIND), and the Epidemiology of Diabetes Interventions and Complications (EDIC). These consortia have collected a large number of samples from patients and families with and without diabetic complications and have plans to release them to interested investigators. One important strategy to validate findings from the human studies will be use of animal models. Candidate genes could be tested for their effects in animals through the use of the Animal Models of Diabetic Complications Consortium (AMDCC) and the Mouse Metabolic Phenotyping Centers (MMPC).

- **Discover genetic modifiers for diabetic complications.**

As genes are identified that impact susceptibility to diabetic complications, a new area of research has emerged that will make it possible to identify genetic modifiers of the clinical manifestation of complications. With the completion of the very dense genetic map known as the International HapMap Project, and new high-throughput genotyping technologies, this promising area of research holds great potential for understanding genetic determinants of the varying clinical severity of diabetic complications. These modifying genes are genetic variants that are distinct from disease susceptibility genes and that modify the phenotypic and clinical expression of the disease genes. Studies show that genetic modifiers can be “tipping

point" genes. This term means that one gene changes the whole phenotype in an all-or-nothing fashion, much like switching a power station "on" or "off." This paradigm contrasts with the incremental effects seen with changes in a large number of non-modifier genes. Many examples of modifier genes are known in humans and model organisms. In fact, the most general lesson learned from experiments with genetically engineered mice may be the profound influence of genetic background on the phenotypic consequences of the engineered variant. Early studies in humans suggest that genetic variants with modifier effects are probably common and diverse in humans.

Because complications are likely to result not only from hyperglycemia, but also from a susceptibility to later pathophysiologic steps, such as inflammation or aberrant angiogenesis, there may be a number of modifier genes relevant to diabetic complications. Discovery of these modifier genes will require integrated studies of different strains of mice, each genetically identical, followed by validation studies in humans. The study of chromosomal substitution strains is a powerful new strategy to discover susceptibility and modifier genes, and to characterize their functional consequences. Accomplishing this research objective will also require careful characterization of patients with type 1 diabetes having either increased susceptibility or increased resistance to diabetic complications.

Animal Models

Despite the remarkable genetic and physiological similarities that are shared between humans and animal models in both health and disease, many other properties are unique to humans. This limitation means that animal models, while enormously informative, are only indirectly relevant to human disease. One of the reasons is that, in comparisons of mouse *versus* human genes, "similar" is not "the same." This distinction means that even subtle differences in gene sequence can lead to functionally important differences in suppression or enhancement of the phenotype by non-conserved amino acids, and responses to potential therapeutic agents may be very misleading.

Research Objective—Develop More Human-like Animal Models of Diabetic Complications:

- **Develop human-like mouse models for diabetic complications.**

A major opportunity in this field stems from cancer research, where mouse models with greater fidelity to human disease are made by substituting critical human genes for the mouse equivalent. Fortunately, significant advances have been made in the genetic modification of animals, so studies to replace rodent genes with human or human-like genes are feasible.

Engineered animal models of diabetic complications with humanized genes will have more direct relevance to questions about complications pathogenesis, and equally important, will have a much greater accuracy in predicting which novel therapies will most likely work in humans. The AMDCC has already produced several new mouse models of diabetic heart, vascular, and kidney disease. It is organized to create better mouse models with relevant human and human-like genes. The MMPCs provide standardized, high-quality metabolic and physiologic phenotyping services for mouse models of diabetic complications.

- **Utilize large animal models of diabetic complications.**

In addition to mice engineered for relevance to human disease, large animal models that more closely resemble human physiology and disease development will also be needed to accelerate the process of "bench-to-bedside" research in the search for new, effective therapies for diabetic complications. For example, inducing diabetes in pigs with the drug streptozotocin provides a model of atherosclerosis relevant to human type 1 diabetes. Validation in such large animal models of potential therapies found effective in mouse models would greatly help to narrow the field of compounds most likely to succeed in human trials.

Biomarkers and Surrogate Endpoints for Clinical Trials

The multi-organ damage caused by type 1 diabetes progresses silently for many years before presenting clinically with the signs or symptoms of disease. It then takes many more years before the occurrence of a well-defined event, such as a heart attack or kidney failure. Detection of early damage to cells and tissue by newly discovered biomarkers is critical for risk stratification of patients, because this early stage of disease progression is likely to be more amenable to treatment. In addition, as tissue damage progresses, the pathophysiologic mechanisms involved in progression are likely to include many more complex elements than those involved in the early initiation phase. This complexity makes therapeutic development more difficult and reversibility of the damage less likely. Biomarkers include the results of a variety of procedures, including laboratory tests, biopsies, clinical testing, and diagnostic images. Development and validation of biomarkers occur over several phases, from discovery of molecular targets or development of new technologies, to testing with patients and controls, to validating results in clinical trials. Examples currently in clinical use include excretion of small amounts of protein in the urine as a biomarker for diabetic kidney disease, exercise echocardiograms as a clinical test for heart disease, and intravascular ultrasound as a diagnostic imaging technique for evaluating atherosclerosis.

Research Objective—Identify Biomarkers or a Combination of Biomarkers for Earlier Detection of Cell and Tissue Damage:

- **Validate newly developed biomarkers.**

Newly developed biomarkers that need further evaluation include: the measurement of intraepidermal nerve fiber density in small skin biopsies as a biomarker of diabetic peripheral neuropathy; and images obtained from non-invasive magnetic resonance imaging (MRI) techniques as a biomarker of diabetic coronary artery disease. Additionally, the development of functional and qualitative assays of endothelial progenitor cells may be useful as biomarkers for cardiovascular risk.

- **Discover specific molecular targets and innovative technologies for early biomarker development.**

Research is urgently needed to optimize measurements of known molecular pathologies such as ROS production and RAGE expression *in vivo*. Discovery of new biomarkers will encompass signature patterns of gene expression or protein expression (genomics and proteomics). In addition, integration of these approaches with novel, non-invasive imaging techniques holds particular promise for evaluating metabolic and pathologic changes over

time. For example, magnetofluorescent, multimodal nanoparticles have been successfully targeted to activated vascular endothelial cells *in vivo* using phage display-derived peptide sequences.

Collaborations among investigators having expertise in complex imaging technologies with investigators having expertise in the molecular cell biology of diabetic complications are likely to produce major advances in this field. These advances will then need to be validated in large clinical trials.

Research Objective—Validate Surrogate Endpoints for Assessing the Progression of Complications in Clinical Trials:

- **Develop surrogate endpoints for clinical trials in diabetic complications.**

At present, the development of therapeutics for diabetic complications is severely constrained because the slow progression rate of complications requires clinical trials of long duration in order to detect changes in outcomes. Surrogate endpoints are biomarkers that are strongly associated with and predictive of disease outcomes. Valid surrogate endpoints can measure the potential of new therapeutics, and be used to provide a strong scientific rationale for longer clinical trials and to prioritize them. They would decrease the risk and improve the planning of clinical trials and thereby encourage development of therapeutics for the complications of diabetes. Developing surrogate endpoints for diabetic complications is an important goal for all the groups involved in drug development, and collaborations between these groups will speed validation and acceptance of new endpoints.

Development of Drug- and Cell-based Therapeutics

Hyperglycemia and the other metabolic effects of type 1 diabetes cause cell and tissue changes that have the potential to be prevented or reversed by treatment. As discussed previously, developing therapeutics that would prevent or reverse diabetic complications is the overarching objective of this chapter. Based on discoveries and data generated from the research objectives outlined in this chapter, the core of this work entails finding agents that will selectively modify molecular targets that initiate the downstream events that lead to diabetic complications.

Research Objective—Identify Therapeutics that Prevent or Reverse the Development and Progression of Diabetic Complications:

- **Use high-throughput screening of molecular libraries to find new therapeutics for diabetic complications.**

The great success of new drug development over the past forty years was based around naturally occurring molecules as leads. Close analogues and derivatives were then designed around these leads. Classical bioassays and biochemistry were used to select compounds that competed with the native molecule for the same active site. Over the past decade, however, this model has been supplanted in large part by a new strategy. This new strategy involves the automated synthesis of large numbers of molecules (called a library) through a process called combinatorial chemistry, and then screening them in rapid biological assays in a process called high-throughput screening (HTS). A demonstration project in screening a library of FDA-approved drugs in assays for diabetic complications is currently underway. In addition, it will be essential to outline and have available critical follow-up mechanisms to

assist investigators in developing lead candidate molecules in the process that leads to clinical trials.

- **Improve the high-throughput assays for diabetic complications.**

HTS has been tried for diabetic complications, but the general consensus is that the HTS assays that have been used poorly simulated the *in vivo* processes involved in diabetic complications. To find effective new drugs, it is essential to create and optimize cell and simple organism-based models of the processes involved in determining the initiation, progression, and regression of diabetic complications. These assays can also be used to test libraries of existing drugs to determine their effectiveness in pathways relevant to diabetic complications. Several of the major research advances described earlier in this chapter have identified specific molecular targets or pathways that need to be pursued as targets for drug development. Other areas still need targets to be identified. One important example is the search for a molecular target that determines the regenerative capacity of diabetic blood vessels and other structures damaged by diabetic complications.

- **Apply the latest advances in drug development technology to diabetic complications.**

Through use of the combinatorial chemistry-HTS approach alone, fewer than expected viable drug candidates have reached the stage of clinical trials. More recently, a complementary strategy to HTS has emerged. This process uses computer-based virtual screening, multidimensional compound property optimization, and *de novo* design of drug-like molecules, which make it possible to identify not just active compounds, but compounds with high potential for optimization into drug-like lead series. While later stages of drug development require the substantial financial resources of pharmaceutical and biotechnology companies, the highly innovative NIH Molecular Libraries Initiative (MLI), a component of the NIH Roadmap for Medical Research, complements the private sector drug discovery effort by creating and screening of a broader range of compounds, and assaying their effects on a broader range of targets. A significant expansion of the MLI into the area of diabetic complications could link innovative and creative academic researchers with the tools and expertise of small molecule discovery and development. This approach would decrease drug development time and greatly improve prospects for clinical success. Mechanisms to enhance interaction between diabetes investigators and scientists leading the NIH Roadmap initiatives would also help facilitate more rapid development of candidate molecules for testing in type 1 diabetes.

- **Encourage the translation to human application of promising new therapies.**

There are a number of critical steps in the translational process for developing new therapeutic agents. Critical for fulfillment of the promise of HTS is that all promising leads undergo careful testing using the best available animal models of diabetes complications. These tests need to be done rigorously and in parallel with validated and consistent outcome measures**.** Similar approaches will be important to move interventions that emerge from fundamental investigation of pathophysiological mechanisms into the clinical arena. Animal studies are a critical prelude to testing the most promising new therapeutic agents in human patients. Given the long timeframe necessary to definitively validate new therapeutic agents in humans, strategies to assess promise of new agents in early phases of clinical testing are

critical. This early clinical testing will likely require the use a panel of existing and new markers for human disease.

Table 5. Key Research Objectives for the Prevention and Reduction of Complications of Type 1 Diabetes

- Identify Molecular Pathways of Hyperglycemia Damage
- Clarify Mechanisms Linking Fuel Utilization and Heart Disease
- Understand the Systems Biology of Diabetic Complications
- Discover the Molecular Mechanisms of Metabolic Memory
- Identify Genetic Factors of Susceptibility and Resistance to Diabetic Complications
- Develop More Human-like Animal Models of Diabetic Complications
- Identify Biomarkers or a Combination of Biomarkers for Earlier Detection of Cell and Tissue Damage
- Validate Surrogate Endpoints for Assessing the Progression of Complications in Clinical Trials
- Identify Therapeutics that Prevent or Reverse the Development and Progression of Diabetic Complications

GOAL VI: ATTRACT NEW TALENT AND APPLY NEW TECHNOLOGIES TO RESEARCH ON TYPE 1 DIABETES

Why This Goal Is Important to People

Type 1 diabetes patients and family members may ask: "How will research help me? Will someone invent instruments to test sugar levels without painful finger sticks? Are there tests that can catch diabetes complications at an early stage?" Simply put, research is the key to a cure for type 1 diabetes. It was through research efforts in the early 1900s that scientists discovered insulin and started using it to save the lives of type 1 diabetes patients. It was also through research that improvements in disease monitoring and treatment strategies have been achieved. These advances have contributed to significant improvements in patients' health and quality-of-life. It will only be through future research efforts that a real cure for this disease will be realized.

Attracting New Talent To Tackle Research on Type 1 Diabetes

Harnessing new and emerging technologies, as well as pursuing all of the future research directions described in this Strategic Plan, is dependent on the existence of a workforce of talented researchers with diverse expertise. It is important to take steps to ensure that creative skilled scientists are attracted to research on type 1 diabetes and its complications and empowered to conduct their research through access to cutting edge tools and technologies.

Recruit Experts in Diverse Fields and Train New Researchers: Because type 1 diabetes affects so many different organ systems (e.g., pancreas, eyes, kidneys, heart, nervous system), and involves such diverse areas of science (e.g., immunology, stems cells and developmental biology, bioengineering) and medicine (e.g., pediatrics, transplant surgery) it is imperative to pursue research on all of these different areas in order to have the greatest impact on the health of patients. Therefore, experienced researchers with a particular expertise (e.g., immunology, heart disease, eye disease) should be recruited to apply their talents to research on type 1 diabetes. Researchers with expertise in many of the newest technologies should also be recruited to apply these sophisticated tools to further understanding of the disease. Moreover, it is important to train and retain new scientists and clinicians to sustain momentum in the field.

Collaborative Research: Increasingly, multi-disciplinary teams of investigators must pool their expertise to catalyze research progress. Because researchers in a particular technology or research area may not have expertise in type 1 diabetes, it is crucial for them to work with scientists who have that expertise. These types of partnerships can truly synergize research efforts and reap tremendous benefits for patients.

Applying New and Emerging Technologies To Type 1 Diabetes Research

The tools of biomedical research have evolved rapidly due to the biotechnology revolution. Many technologies that were used 20 years ago have been replaced by new technologies that permit scientists to conduct research more efficiently and to ask and answer questions that they previously could not even begin to frame. Some new and emerging technologies hold real promise for advancing the type 1 diabetes research field.

Visualizing Islets in the Body: When a person breaks a bone, an x-ray is used to see the injury. This technique makes it much easier for doctors to diagnose the break and effectively treat it. Likewise, it would be useful to "see" a person's islets. Why would this be important? Type 1 diabetes is usually diagnosed late in the disease process, when most of the insulin-producing beta cells have already been destroyed. If researchers could detect islet destruction by visualizing the islets before the onset of clinical symptoms, then they could intervene earlier to try to prevent further islet loss and the need for insulin administration. Furthermore, such a technological breakthrough would make it more efficient to conduct clinical trials, because scientists could actually "see" if a therapy was effective at either preventing or reversing islet loss. Another potentially beneficial application of this technology is to improve outcomes of islet transplantation. If doctors could see when transplanted islets are beginning to be rejected by the immune system, then they could intervene earlier in order to prevent graft rejection and the need for patients to resume insulin administration. Recently, significant advances in visualizing islets using techniques such as magnetic resonance imaging (MRI) have been achieved in mouse models. More research in this area is needed to translate these results to humans to overcome this major clinical and research barrier in type 1 diabetes.

Technology for Identifying Disease Genes: With the completion of the Human Genome Project and with new "high-tech" laboratory methods, genetics experiments that used to take months now take minutes. These advances will significantly speed the discovery of disease-causing genes, including those that play a role in type 1 diabetes disease onset or the development of complications. Identification of key genes will promote the development of novel prevention strategies.

Studying Proteins Involved in Disease Onset and Progression: Recent research advances have improved scientists' ability to study proteins in the body. Fifteen years ago, researchers were only able to study relatively small numbers of proteins at one time to determine if or how they had a role in disease onset or progression. Because there are tens of thousands of proteins in the body, studying proteins a few at a time is an extremely time-consuming endeavor. However, novel "proteomics" technologies have been developed that now permit researchers to study thousands of proteins at once, as well as to determine how proteins may interact with each other. These technologies can be used, for example, to identify proteins that correlate with stage or rate of progression of type 1 diabetes and its complications. Furthermore, understanding the expression and function of proteins will enhance understanding of the genes by which the proteins are produced. These insights could directly translate to improved disease detection and prevention strategies.

Application of Engineering Principles: There are several areas of type 1 diabetes research that could benefit from the application of engineering principles to disease ("bioengineering"). For example, identifying ways to measure blood glucose levels without the need for a "finger stick" would dramatically improve the quality-of-life of type 1 diabetes patients. Even more beneficial would be linking such measures to insulin delivery devices to create an "artificial pancreas." In the field of islet transplantation, if transplanted cells could be protected from the immune system by some material or device, then there would be a higher chance of transplant success with avoidance of toxic immunosuppressive drugs. To realize these and other advances, it is important to apply bioengineering approaches to type 1 diabetes research.

Animal Models To Study Type 1 Diabetes: Animal models are an important scientific resource because they enable researchers to investigate underlying disease processes that cannot be studied in humans. These models also permit assessment of novel therapeutic interventions before they are tested in people. The use of animal models is a necessary early step to promote translation of research findings from the laboratory to human patients. It is crucial to develop and utilize animal models with greater fidelity to human type 1 diabetes and its complications to propel research progress.

Gene Therapy Approaches: When genes in the body are defective, a plausible treatment strategy is to replace them with those that work properly. Researchers have been exploring novel ways to deliver genes to people or transplanted tissues, a process called "gene therapy," and these approaches, once developed, could also be used to benefit type 1 diabetes patients. For example, islets transplanted into a type 1 diabetes patient undergo attack by the immune system which treats them as foreign invaders. Gene therapy approaches could be used to protect islets from this attack or to deliver genes which enhance islet viability in the transplant site. With future research and scientific breakthroughs, gene therapy approaches could also be used to treat diabetic complications, as well as to replace the insulin that type 1 diabetes patients are no longer capable of producing. Gene delivery approaches are also being used to create animal models for study of therapies for type 1 diabetes and its complications.

Collection and Analysis of Scientific Data: Because scientists are now collecting more data than they ever thought possible, it has become increasingly important to find ways to assemble, organize, and analyze this valuable information. Furthermore, in order to achieve the greatest impact on the field, scientists must be able to share data with one another, so that they can compare results or combine their efforts to make novel discoveries that they cannot make individually. It is important for researchers who are conducting different studies to work together and coordinate their efforts in order to accelerate the pace of discovery.

Moving the Research Agenda Forward

New and emerging technologies, coupled with a cadre of talented scientists, have the potential to bring about real breakthroughs in the understanding, prevention, treatment, and cure of type 1 diabetes. Under the auspices of the *Special Statutory Funding Program for Type 1 Diabetes Research*, multiple consortia have been created to tackle specific challenges that will impact the health of people with type 1 diabetes. These efforts bring together clinical and basic researchers, and link scientists investigating the pathogenesis and therapy of type 1 diabetes and its

complications with new technologies needed to pursue evolving areas of opportunity. New therapies have already had a dramatic impact in extending life and retarding disability from type 1 diabetes. The pace of discovery is accelerating and, as in the past, future research advances should directly translate into improvements in the health and quality-of-life of patients. Therefore, it is crucial to deploy new and emerging technologies and engage experts in diverse areas in the battle to overcome type 1 diabetes and its complications.

This is a new and exciting era of scientific research. Scientists are now able to study biologic processes in ways that were not possible even a few short years ago. It is essential to take full advantage of the new technologies and information that have emerged in order to optimize progress. A talented workforce of researchers must be mobilized to apply their expertise to overcome current barriers. Type 1 diabetes is a devastating illness for patients and their families, especially when it strikes in infancy, childhood, or adolescence. Pursuing novel research directions and attracting new research talent are key elements in conquering this disease.

GOAL VI: ATTRACT NEW TALENT AND APPLY NEW TECHNOLOGIES TO TYPE 1 DIABETES RESEARCH

INTRODUCTION AND BACKGROUND

In the last 100 years, doctors and scientists have made remarkable progress in the understanding and management of type 1 diabetes. As a result, people with the disease are now living longer and healthier lives. As the quest for a cure continues, progress will increasingly require collaborations among clinical and basic scientists with diverse skills and expertise. To understand the complicated interplay of hereditary and environmental factors that cause the disease and the progression of its complications (Goals I and V), geneticists and epidemiologists are beginning to collaborate with biostatisticians and informational biologists to generate computer models that will allow them to understand and test these complex interactions in the biological system. Preventing and reversing the chain of events in autoimmunity and achieving immune tolerance for organ transplants (Goals II and III) will require cooperation between immunologists and clinicians, as well as the biotechnology industry that develops and tests therapeutic agents. Similarly, knowledge of the basic biology of the insulin-producing pancreatic beta cell (Goal III) has expanded because of efforts to recruit to the study of diabetes cell and developmental biologists who may have been focused on other systems and diseases. These talented scientists are now concentrating their skills toward understanding how the beta cell develops. The research challenge of hypoglycemia unawareness (Goal IV) will require recruiting more neurobiologists and endocrinologists to understand the brain circuitry and body interactions. Continuing the progress that has been made in early detection and slowing progression of diabetes complications (Goal V) will involve not only experts in heart, nerve, kidney and eye disease, but also expertise in proteomics, imaging, and other skills needed to develop biomarkers and surrogate endpoints that can speed translation of new therapeutic concepts from the bench to the bedside. For each of the Strategic Plan's scientific goals, a crucial pre-requisite is to recruit and retain talented scientists and to foster collaborations, in order to propel further scientific advances.

With the recruitment of appropriate talent to the study of type 1 diabetes, cutting-edge technologies can be applied or developed for use in basic and clinical research. Certain technology themes cut across all the research goals and objectives outlined in this Strategic Plan. For example, the application of biophysical tools, such as labeled tracers, has opened the door for the use of new and improved methods of non-invasive imaging in both patients and animal models. These techniques will allow clinicians to assess the onset and progress of disease and the success of various therapeutic interventions. In recent years, biomedical research has witnessed an explosion of innovative tools that have paved the way for new fields of research such as proteomics, functional genomics, metabolomics, bioinformatics, gene therapy, and gene silencing (siRNA). Scientists are rapidly applying these new technologies to type 1 diabetes research. However, it would also be beneficial to design additional new technologies in the context of type 1 diabetes research so as to address the unique challenges of this disease. Additionally, new technologies may facilitate identification and validation of improved biomarkers for disease progression. Such biomarkers would make it less expensive and more

efficient to conduct clinical trials and would thereby encourage industry investment in new therapies and enable patients to benefit more quickly.

RECENT SCIENTIFIC ADVANCES

Novel Imaging Technologies To Monitor Type 1 Diabetes Disease Progression and Islet Transplantation: Type 1 diabetes is usually diagnosed very late in disease progression, when most of the insulin-producing beta cells of the pancreas have already been destroyed by the immune system's misguided attack on its own tissue (autoimmunity). Needed is a toolbox of imaging technologies to detect the first signs of beta cell destruction, to monitor therapy against immune attack, or look for possible regeneration of beta cells. The first steps have been taken; scientists have recently developed a new, non-invasive imaging technology to monitor infiltration of inflammatory cells into the pancreas in an animal model of type 1 diabetes. This approach is now being tested in people. If successful, it could dramatically improve the ability of researchers to perform type 1 diabetes clinical trials.

Another important advance is the successful labeling of isolated human and mouse islets, and mouse T cells, with non-toxic imaging probes that can be detected with Magnetic Resonance Imaging (MRI), fluorescence, or nuclear imaging. The islets have been imaged quantitatively over time after implantation in the liver or under the kidney capsule in mice. T cells have been seen as they infiltrate the pancreas of a NOD mouse. Although such molecular imaging approaches are still very new, their application is starting to be introduced into human patients and it is hoped that these will soon include studies in type 1 diabetes.

Scientists are also exploring the use of Positron Emission Tomography (PET) imaging to see radiolabeled ligands targeted to the pancreas. If such an approach proves successful, it would allow physicians to estimate the number or mass of a patient's own endogenous beta cells as well as to monitor the fate of transplanted islets.

Systems Biology Approaches Reveal Identity of Genes involved in Pathophysiology of Diabetes: Some diseases are caused by changes in the DNA of a single gene leading to a defective or missing protein, but complex diseases may involve subtle changes in the concentrations of a whole network of proteins working in concert. These changes can often be detected as a function of the concentration of the mRNA molecules that arise from DNA and code for proteins. DNA microarrays are a powerful tool that permit geneticists to simultaneously monitor the changes in gene expression (mRNA) of an entire genome to compare healthy and diseased tissues. Computational scientists are now working with biologists to develop adequate tools to analyze the vast amounts of data produced in each experiment in order to more fully understand its value. Bioinformaticists recently introduced just such an analytical strategy that enabled them to compare gene expression in muscle biopsies from diabetic and non-diabetic individuals. Their analysis allowed them to identify a set of genes whose expression is coordinately decreased in diabetic muscle. This group of genes carries out energy production in mitochondria, structures often referred to as "the powerhouse of the cell." The affected mitochondrial protein genes are controlled by two transcription factors—therefore, the few genes that code for these special transcription factor proteins regulate the expression of many other genes. Rare forms of monogenic diabetes, such as the *Maturity Onset Diabetes of the Young*

(MODY), provide another example of the key role of selected transcription factors in the pathogenesis of diabetes. These disorders originate from mutations in key transcription factors that affect entire networks of genes that regulate function in organs such as the liver and pancreas. Using antibodies raised against a transcription factor known to be involved in pancreas development and liver metabolism, scientists can isolate all of the regions of the genome that bind to that transcription factor (chromatin immunoprecipitation or ChIP). The sequence of the DNA regions binding to the transcription factor of interest can then be identified using either large scale sequencing strategies (Serial Analysis of Chromatin Occupancy or SACO) or hybridization on promoter microarrays containing the promoter regions of all known genes (ChIP-on-chip). These genome-wide analyses can result in fast, complex, and accurate modeling of transcriptional regulatory networks involved in the control of energy homeostasis, pancreatic beta cell function, or pancreatic islet mass.

miRNA Involved in Regulation of Insulin Secretion: A novel class of natural molecules that controls translation of specific proteins was recently discovered and characterized. These are microRNAs (miRNA)--small single stranded chains of nucleic acid derived from non-coding portions of the genome. Building on this new discovery, researchers have recently found a miRNA in mice that suppresses insulin secretion. Using a miRNA-related technology called RNA interference (RNAi—see accompanying sidebar), the researchers were able to mimic the effects of the miRNA to regulate insulin secretion. Furthermore, *in vivo* use of chemically engineered oligonucleotide inhibitors of miRNA, called "antogomirs," indicate that a single miRNA is likely to have not one but many gene targets. Antagomirs are therefore powerful tools that can silence *in vivo* miRNA-controlled regulatory pathways and could become a therapeutic strategy for pathologies in which miRNAs participate in disease etiology.

Sidebar— Potential Therapeutic Applications of RNA Interference (RNAi) in Type 1 Diabetes

The recent discovery of the natural molecules known as microRNA (miRNA) has challenged the prevailing scientific thinking regarding the role of ribonucleic acid (RNA) in gene regulation. The miRNA has much shorter chains of nucleic acids than messenger RNA (mRNA), which contains the coding sequences of proteins. These miRNAs can specifically silence the expression of a gene or a family of genes by blocking translation of the proteins they encode, and are involved in the regulation of a wide variety of cellular functions, ranging from cell-fate determination to suppression of glucose-induced insulin secretion in the beta cell. Mammalian genomes contain a large and diverse family of miRNAs; it is now believed that miRNAs, conserved across evolution from plants to animals, may affect one-third of all human gene expression. However, because scientists have spent decades focusing on protein-based mechanisms of gene regulation, the research tools for studying miRNA regulation are just now being developed.

Researchers have learned how to manipulate the pathways used by miRNA with synthetic double-stranded RNA molecules called small interfering RNAs (siRNA). These are employed in RNA silencing or interference studies (RNAi) to suppress translation of the gene of interest and provide insight into its function. Successful *in vivo* delivery of siRNA has been demonstrated in a wide variety of organs, including pancreas. This technology is quickly moving into the clinic,

as several biotechnology companies have received FDA approval for conducting phase 1 clinical trials using RNAi-based therapies. In one such phase 1 trial, siRNA technology is used to target the vascular endothelial growth factor (VEGF) pathway to treat age-related macular degeneration; visual acuity improvement has already been reported in the treated group. If successful, such an approach may be used for the treatment of diabetic retinopathy resulting from abnormal, VEGF-dependent angiogenesis in the retina. Other RNAi treatments in development are targeting specific immune and inflammatory responses. For example, a program using siRNA targeting key Th2 cytokines is entering a phase 1 trial for the treatment of asthma. RNAi-based therapeutic strategies targeting specific components of the immune response and/or the apoptotic pathways involved in beta cell destruction, combined with early detection of disease, could result in the restoration and maintenance of normal beta cell mass in type 1 diabetes patients.

Brain Imaging in Hypoglycemia and Unawareness: The brain is dependent on glucose for fuel, and also contains glucose-responsive neurons in specialized regions that sense blood glucose concentration and then coordinate the hormonal, neurological, and behavioral responses that rescue a person when his or her blood glucose levels sink too low. Diabetes can be accompanied by a failure of the brain to recognize the low blood sugar (hypoglycemia), and to generate signals to prevent blood sugar from falling to a dangerously low level. This hypoglycemia unawareness often develops after repeated episodes of hypoglycemia and is very frightening to diabetes patients who may lose consciousness without warning. Now, scientists are beginning to understand how the brain loses its ability to respond and to warn of impending hypoglycemia. New imaging technologies have revealed differences in glucose use in different areas of the brain. Imaging has also shown that the brain increases blood flow when blood glucose falls, and that its cells store glucose that can be used when it cannot get enough from the blood. Combined, these mechanisms may help maintain glucose availability to the brain, and thus cognitive function, when the body experiences hypoglycemia. Most people with diabetes have the same brain glucose concentrations as healthy people, but brain glucose is elevated in those who develop hypoglycemia unawareness; thus glucose levels may stay higher in their brain cells even as blood glucose levels fall, masking the normal warning to respond to low blood glucose, and therefore failing to prevent it. Considerable progress remains to be made in this area before achieving a true understanding of how the brain reacts to glucose concentrations and what goes awry in hypoglycemia unawareness in diabetes. However, the new imaging technologies of Magnetic Resonance and Positron Emission Tomography have opened the window on the brain and its metabolism.

Engineering an Endless Source of Beta Cells for Therapy: The limited supply of human islets made available for transplantation dramatically reduces patient access to this potential life-enhancing therapy. In laboratory studies, efforts to produce an increased quantity of glucose-regulated, insulin-producing cells for transplant have employed cell culture, tissue engineering, and gene therapy technologies to promote beta cell development from adult or embryonic stem cells. In addition, mature cells from tissues such as liver, spleen, intestine, or the pancreas, or from cultured cell lines, have been tested for their ability to serve as donors in cell-based therapies. To enhance their therapeutic potential, these cells have been incubated with growth and transcription-activating factors or hormones, co-cultured with additional cell types, or been the recipients of genes that code for proteins found in the beta cell. Insulin-secreting beta cell-

like immortalized cell lines have been created by careful selection for beta cell specific traits. These efforts have yielded cells that, in some cases, are able to reverse diabetes in animal models, but have not yet been fully successful in reproducing the exquisite sensitivity to small changes in glucose levels characteristic of mature beta cells. This research has also provided considerable insight into the characteristics that make a beta cell what it is, and provide a foundation for further progress toward improving the supply of beta cells or islets.

RESEARCH OBJECTIVES AND STRATEGIES TO ACHIEVE GOALS

Engaging Talented Scientists

Pursuit of the full range of opportunities for prevention and improved therapy of type 1 diabetes and its complications requires a wide range of scientific expertise and the participation of investigators from diverse fields. These talented mature researchers must be recruited to the field, and promising new investigators must be trained in diabetes and enlisted in the research enterprise. High-yield multi-disciplinary approaches can only be fostered with appropriate new infrastructure. Finally, resources must be provided to promote an environment that values high-risk, high-impact projects. Tomorrow's ability to prevent and cure type 1 diabetes depends on the quality of today's research community and environment.

Research Objective—Recruit Expertise from Diverse Fields:

- **Encourage interdisciplinary collaborations.**

Type 1 diabetes affects many different organ systems (e.g., pancreas, heart, kidney, eye, nervous system) and requires expertise in fields as diverse as genetics, neurobiology, immunology, biophysics, endocrinology, imaging, bioengineering and biostatistics. The NIH has pioneered novel approaches to establish and empower new scientific teams for type 1 diabetes research.

Collaboration among scientists with complementary expertise has been forged via "Innovative Partnerships" (see accompanying sidebar) that encourage experts in type 1 diabetes research to recruit and work together with scientists from outside the field. In "bench-to-bedside" research partnerships, a team of clinical and basic scientists conducts collaborative research that, if successful, will bring basic research advances from the laboratory to a point where a potential new therapy can be tested in patients or in pre-clinical studies in animal models. A major obstacle to research is being alleviated by programs that provide scientists with access to collections of biologic samples from well-characterized, consenting patients. The repositories that store and distribute such samples are an important resource that allows the creative research community to conduct mechanistic studies in virtual collaboration with the clinicians as they pursue patient-oriented research.

Scientific workshops bring together experts from different fields to stimulate discussions and cooperative endeavors in a particular field, and have resulted in important new collaborations. For example, one such key workshop brought together clinical researchers from the landmark Diabetes Control and Complications Trial with cell biologists to explore the mechanisms underlying "metabolic memory" (see Goal V). Another workshop drew together neuroscientists and diabetes experts to address the problem of hypoglycemia

unawareness that limits therapy for type 1 diabetes (see Goal IV). Attendees outlined collaborative strategies to elucidate the mechanisms underlying this condition and explore avenues to reverse it. A third workshop brought together proteomics experts and researchers in the fundamental and clinical aspects of diabetes research, and yielded valuable suggestions for future efforts to develop much needed biomarkers and surrogate outcomes.

Sidebar—Innovative Partnerships in Type 1 Diabetes: A Novel "Co-Principal Investigator" Support Mechanism

Because research on type 1 diabetes spans a broad range of scientific disciplines, propelling research progress requires a cadre of scientists with diverse research training and expertise. To attract new research talent to study type 1 diabetes and its complications, the NIH has supported an initiative on "Innovative Partnerships in Type 1 Diabetes Research." The overall objective of the initiative was to support collaborations between investigators who focus their research efforts on type 1 diabetes or its complications and investigators from other research areas with expertise relevant to type 1 diabetes. Type 1 diabetes researchers therefore acted as "talent scouts" by identifying and recruiting leading scientists with scientific expertise relevant to the field of type 1 diabetes research. Using this mechanism, researchers with expertise in areas such as cell-based screening, imaging, genomics, and systems engineering are now pursuing research on type 1 diabetes.

The intent of the initiative was to encourage true partnerships in which two or more investigators with complementary expertise tackled a common problem. However, the standard policy at the NIH was to award an actual grant to only one "Principal Investigator," while the "partner" was listed as a "co-Investigator"--an arrangement that did not recognize both partners as being "equal" and thus posed a barrier to collaboration. Based on feedback received from the external scientific community, the NIH pioneered a novel solicitation so that both partners were named as co-equal principal investigators. This arrangement was first used under the *Special Statutory Funding Program for Type 1 Diabetes Research.* It provided an important incentive to collaboration, and attracted expertise from diverse fields. For example, one project brought together diabetes complications investigators with experts in angiogenesis (small blood vessel formation), thereby helping to move therapeutics currently used for cancer toward applications for diabetes complications. The new awards benefited both partners, who have now received equal recognition for their contributions to the research study. This recognition can be beneficial to investigators, who may be evaluated by their home institution in terms of the number of grant awards they have received. The "co-principal investigator" mechanism--first employed by the NIDDK with the innovative partnership for type 1 diabetes initiative--is now being considered for broader implementation by the NIH as a whole, under the NIH Roadmap for Medical Research.

- **Promote "high-risk, high-impact" research.**

Scientists have identified certain barriers as critical bottlenecks that impede progress in type 1 diabetes. Particularly noteworthy is the need for biomarkers and surrogate endpoints to conduct clinical research to evaluate potential new therapeutics in small pilot trials. However, this type of discovery research is inherently risky, with no assurance of a positive outcome. Moreover, applicants find it difficult to obtain funding for high-risk research even

if the work has potential for high, positive impact. Although not all high-risk research makes it to the clinic, investment in pioneering research may eventually stimulate the next major breakthrough. Therefore, it is imperative to provide incentives for talented scientists to undertake such research. These can take the form of limiting the requirements for preliminary data for pilot studies, and of providing quick turn-around for continued or expanded funding when pilot studies meet defined milestones for achievement.

- **Create an environment conducive to innovation and collaboration.**

Although science has traditionally been fueled by competition, recent efforts focused on aspects of type 1 diabetes research have attempted to alter that paradigm and foster a community-oriented approach through the establishment of large research consortia, clinical trial networks, research centers, and team science. Such cooperation has enabled researchers to undertake large interdisciplinary projects that could not be pursued independently by any single investigator or small research group. Within a consortium, team members can share data, samples, protocols, research resources, and even cost-intensive patient recruitment in an efficient and effective way. The *Special Statutory Funding Program for Type 1 Diabetes Research* has not only created many effective consortia, but it has also recently provided support for infrastructure to promote cooperation among consortia. A consortia coordinating committee has been formed to resolve issues such as interoperability of databases and standardization of patient informed consent procedures. Furthermore, the NIDDK supports a website to announce the availability of research resources and funding opportunities to the research community (accessed at: www.T1Diabetes.nih.gov/investigator).

- **Attract and train new diabetes investigators.**

New scientists and engineers often bring energy and creativity to a field; future progress depends on the research training and mentoring of new students, post-doctoral fellows, and independent investigators. Currently, the *Special Funding Program* provides competitive institutional research career and training awards for pediatric endocrinologists involved in type 1 diabetes research (see accompanying sidebar). While development of pediatric endocrinologists as diabetes researchers was considered the highest priority for the limited resources available, this program could be productively expanded to promote research career development and training for investigators in other areas of importance to type 1 diabetes research. Programs that provide funding for exploratory projects could be very influential at the critical time that junior investigators are making the choices that will determine their long-term career paths.

Sidebar--Training Clinicians for Diabetes Research

To enlarge the pool of pediatric endocrinologists conducting diabetes research, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), in partnership with the American Diabetes Association and the Juvenile Diabetes Research Foundation International, awarded institution-wide research training and career development grants to seven medical centers with strong research programs in childhood diabetes (www.niddk.nih.gov/fund/diabetesspecialfunds/train_peddiab.htm).

The awards provide for fellowship training as well as support for junior clinical investigators, for a total of 5-6 years of continuous, uninterrupted research training and career development in

childhood diabetes. These T32/K12 awards currently support 34 pediatric endocrinology fellows/junior clinical investigators each year who are receiving training and career development in many aspects of diabetes research. Thus far, nine pediatric endocrinologists supported by this program have received individual NIH or JDRF career development awards. Moreover, more than five of the trainees were recipients of an award through the NIH Loan Repayment Program to enable highly promising new clinical researchers to offset some of the educational debt incurred during their graduate education in the health professions (www.lrp.nih.gov).

Development and Application of New Technologies

The past decade has seen major advances in biotechnology with direct relevance to type 1 diabetes research. It is imperative to put cutting-edge technology into the hands of type 1 diabetes researchers and to foster future development of these tools in the context of their application to type 1 diabetes. Highlighted below are some of the most promising new technologies with important applications for type 1 diabetes.

Research Objective—Establish Non-invasive Imaging Technologies for Type 1 Diabetes Research and Diagnostic Applications:

- **Develop imaging for pancreatic beta cell mass, function, and inflammation.**

As discussed in the "Recent Scientific Advances" section of this chapter, there has been impressive progress in this area despite substantial challenges inherent in visualizing a tiny population of cells that reside deep inside the abdomen and share many defining characteristics with neighboring cell types. Success in imaging will provide insight into the natural history of islets in diabetes; facilitate clinical trials to test therapies to slow or reverse beta cell loss; and allow physicians to monitor engraftment following islet transplantation. An islet imaging program funded by the NIH and JDRF has supported promising projects in animals and in humans looking at original surviving islets, as well as transplanted ones (see accompanying sidebar). Parallel projects are ongoing to provide the important reagents for imaging. These include the identification of unique cell surface proteins and production of monoclonal antibodies and other specific ligands that can be tagged for molecular imaging. The next steps are to create an environment in which various imaging approaches and reagents can be translated from the laboratory to clinical application in a well-characterized patient population. This transition will require a balanced team of cutting-edge physicians, imaging experts, chemists, and biologists who have access to state-of-the-art equipment for imaging in both animal models and patients. Finally, entirely new approaches are being designed for imaging live tissues. These include:

- New optical tools that are more sensitive to small differences among tissues, can image deeper into the body, or can take advantage of optical fibers and miniaturized detectors that can be introduced into the body in a mildly invasive manner;
- New x-ray imaging that can see soft tissues with very high resolution and reduced radiation exposure;
- New highly sensitive ultrasound and MRI contrast agents; and
- Enhanced resolution through powerful image reconstruction paradigms.

The diabetes research community must be positioned to take advantage of the best of these technologies as they appear, so that they can immediately be brought to bear on the challenging problem of imaging the pancreatic islet beta cell.

Sidebar--Imaging: An Inside Look

Seeing is believing. Imaging scientists are working to find ways to visualize the processes that lead to diabetes, and how the body responds to therapy. These new tools will further a better understanding about how the disease starts and progresses. Imaging techniques will provide insights into why, how, and when diabetes occurs, as well as point the way to new ways for treating the disease.

The secret to imaging diabetes is the use of drug-like imaging agents that selectively "light up" the cells or biological processes involved in disease. For instance, the metals iron and gadolinium change the signal in Magnetic Resonance Imaging (MRI). Compounds that contain these metals can be designed to home in specifically on the insulin-producing beta cells in the pancreas, and permit them to be counted. Similar compounds have been used to light up the inflammation in the pancreas that accompanies the autoimmune destruction of the beta cells and therefore causes type 1 diabetes. Other imaging agents mimic nutrients or hormones, and when taken up by cells, reveal clues to their function and metabolism. These types of agents are commonly labeled with minute levels of radioactivity and detected by Positron Emission Tomography (PET). Thus, they might allow researchers to distinguish among active and distressed beta cells. Currently, considerable effort is focused on putting imaging labels on the isolated pancreatic islets used for transplantation into diabetic patients. This approach would enable doctors to actually watch the locations to which these tissues migrate once they are infused into patients, and to determine their fate—that is, to know how many survive to produce insulin, find out whether they grow in their new environment, and see what happens to those that die. Imaging might also disclose the formation of new blood vessels and nerves around the islets, and pinpoint the importance of these processes for insulin secretion.

Scientists have learned to incorporate into mice a family of proteins that either emit light (such as the luciferase/luciferin system from the firefly) or fluoresce (such as green fluorescent protein). These constitute a very powerful set of imaging tools that are used in basic animal research. For instance, fluorescently labeled insulin can be tracked by the microscope to uncover defects in insulin secretion that might be involved in diabetes. It is hoped that these tools will help researchers identify and monitor a precursor cell that can become a new insulin-producing beta cell.

Imaging may one day help manage diabetes or identify patients prone to diabetic complications before they become clinically obvious. For instance, new glucose-sensitive imaging agents may make possible the continuous monitoring of plasma glucose without fingersticks. Such an advance would be enormously beneficial for patients. Scientists are therefore working to bring emerging imaging tools to bear on all aspects of diabetes and its treatment.

- **Develop brain imaging techniques to understand hypoglycemia.**

How do minutes or hours of hypoglycemia affect structure, function and metabolism of the brain? What are the short and long-term consequences of multiple episodes of low blood sugar? What leads to hypoglycemia unawareness? To answer these questions, improved brain imaging with very high spatial resolution is needed to elucidate the relationship between the specific neurons and their supporting cells involved in the detection and response to low blood sugar. It will likely be necessary to introduce artificial molecular imaging tags that bind to specific surface proteins in order to distinguish cell types from one another in glucose-sensing regions of the brain (see sidebar on *Imaging: An Inside Look*). The ability to visualize neural function is needed to understand hypoglycemia unawareness. There are several novel functional imaging technologies being used to study the brain, such as BOLD fMRI, arterial spin labeling techniques, diffusion tensor imaging, and magnetoencephalography. Because of the relatively small brain regions involved, the ability to study hypoglycemia may benefit from an investment in additional novel functional imaging tests. In addition to technology, experimental paradigms are also needed that couple physiological responses measured by imaging to reliable measures of behavior in response to hypoglycemia.

Research Objective—Promote Application of Advances in Bioengineering to Type 1 Diabetes:

- **Develop novel drug delivery methods.**

Effective drug delivery depends on applying the proper dosage in the proper location over the proper time course, while overcoming issues of target specificity and drug degradation. Bioengineered drug-eluting polymers can be implanted to slowly release a drug over time directly at the site where it is needed, such as in the eye to treat diabetic retinopathy, or directly in a foot ulcer. Cardiac stents can be coated with drugs that locally suppress immune reactions or prevent reocclusion of the vessel. Scientists are also embedding immune suppression drugs or compounds that promote angiogenesis or islet replication into materials used to encapsulate and protect islets for transplant.

- **Develop non-invasive glucose monitoring technologies.**

An artificial pancreas would require a continuous glucose sensor whose output could be used to regulate an insulin pump in a feedback loop. To achieve such a closed loop system, glucose monitors are needed that are faster, more accurate, and easier to use. Flexible algorithms are needed to link the changes in blood or interstitial fluid glucose to insulin delivery. Although the artificial pancreas is not yet available, the first steps have been taken. The NIH supports basic sensor research in universities and industry, as well as clinical assessment of devices arising from these projects by independent academic investigators.

- **Integrate tissue engineering and regenerative medicine to develop tissues and organs to replace those destroyed by diabetes and its complications.**

Tissue engineering is an exciting emerging field in which biocompatible synthetic polymers, cells, and tissues, gene manipulation technology, drugs, and natural biological molecules are all brought to bear. Bioengineered tissues could improve therapy for diabetes complications: artificial skin for the repair of diabetic foot ulcers, heart muscle patches, and improved vascular access for dialysis. Glucose-responsive, insulin-secreting cells engineered from a readily available cell source may replace the human cadaver islets that are currently being

used for transplantation and are in short supply. If a patient's own cells could be used as the precursor, this may alleviate the need for immunosuppressive drugs and greatly increase the number of patients who could be treated.

- **Apply nanomedicine to drug delivery, islet encapsulation, non-invasive imaging, and glucose sensing technologies.**

The Office of Science and Technology Policy in the Executive Office of the President has launched a government-wide initiative to invest in the burgeoning field of nanotechnology. Nanotechnology is the manufacture, study, and use of molecules with unique properties when observed at a nanoscale level--larger than atoms but much smaller than cells. This is a fertile field in which to engage engineers and scientists to work on the technologic challenges described above in type 1 diabetes research, including: imaging, tissue engineering, drug delivery, immunoprotective coatings, and development of an artificial pancreas.

Research Objective—Foster Application of Gene Delivery and Gene Silencing Technology To Develop New Therapies for Type 1 Diabetes and its Complications:

- **Develop technology for gene delivery to cells and tissues that are therapeutic targets for type 1 diabetes.**

Gene therapy is an experimental approach to introduce into cells a gene into cells that either replaces a mutated, disease-causing gene or provides a new cellular function. Complications such as diabetic retinopathy, neuropathy, and wound healing are potential targets for gene therapy. Instead of introducing vectors globally into the whole body, vectors could be applied directly to the affected site, which should reduce toxicity. Gene therapy has been used in an animal model to deliver growth factor genes to skin ulcers and has been successful in accelerating wound healing. Based on these studies, a human trial using this approach to improve wound healing has been initiated. Gene therapy applications are also being tested in animal models to deliver genes to the retina or to nerve cells to prevent cell damage. Gene therapy using viral vectors has been successful in treating another disease of the retina, retinitis pigmentosa, in a dog model. Despite the fact that this technology is still at a very early stage of development, these applications to diabetic complications are being actively pursued.

- **Create siRNA vectors for gene silencing in target tissues.**

As described in the accompanying sidebar, siRNA is a new technology that allows researchers to efficiently and rapidly reduce the level of expression of proteins in cells and tissues. Used as a research tool, *in vitro* and *in vivo*, siRNAs enable researchers to better understand the contribution of specific proteins to regulatory or disease pathways. For example, using siRNAs to specifically silence disease-causing genes in NOD mouse models will help geneticists dissect the particular contributions of these genes to the development of a diabetes phenotype. Similar techniques will allow immunologists to understand co-stimulatory pathways that control the balancing of immune cell function (effector and regulatory T cells). In addition to being used in basic science, siRNAs are being combined with gene therapy vectors to silence a variety of genes involved in disease onset and complications in patients.

Research Objective—Exploit the Potential Application of New and Emerging Technologies in Functional Genomics, Proteomics, and Metabolomics to Type 1 Diabetes Research:

- **Use "omics" technologies to identify interactions among genes, proteins, and metabolites in type 1 diabetes and its complications.**

Functional genomics is the new field of science that employs DNA microarrays to measure those genes that are active in a tissue under a given set of conditions, and identifies clusters of genes that work together. Human and mouse "PancChip" microarrays were developed by the Beta Cell Biology Consortium (BCBC; see Goal III) and contain thousands of genes expressed specifically in the pancreas. This resource is critical for identifying the pathways involved in the development and function of the beta cell and is being distributed widely to investigators seeking to develop an unlimited supply of beta cells for cure of diabetes. The PancChip has significant importance for other diseases as well, such as pancreatic cancer.

The function of a gene is fulfilled through the proteins whose formation it directs. The complete set of proteins and their interactions, or "proteome," provides further opportunities for systematic analysis and exploration. Proteomics involves the use of several novel integrated technologies to identify and quantitate proteins and study their interactions, modifications, and dynamics. Proteomic technologies have been successfully used for the identification of cancer biomarkers, the elucidation of biochemical pathways, and the pinpointing of novel drug targets. "Metabolomics" studies the small molecules such as amino acids, carbohydrates, and lipids in the cells, tissues and biofluids of an organism. The "metabolome" responds quickly to disease, diurnal and nutritional variation, and can be a very sensitive indicator of a person's current metabolic state. Large-scale approaches, such as proteomics, genomics, and metabolomics, are promising technologies for understanding the complex molecular mechanisms that underlie type 1 diabetes and its complications.

- **Utilize proteomic and metabolomic technologies to identify and validate surrogate markers that predict risk, rate of progression, or response to therapy for type 1 diabetes and its complications.**

A current major barrier in conducting type 1 diabetes clinical trials is the need for easily measured biomarkers that adequately predict disease risk and progression well before a measurable clinical outcome such as diabetes onset, or a serious complication such as heart attack. "Omics" technologies will provide "fingerprints" or patterns of molecules diagnostic of disease that may be more powerful as clinical biomarkers than a single molecule such as glucose or glycosylated hemoglobin. Many events that clinicians would like to monitor do not have such a single marker, such as the autoimmune process of type 1 diabetes or the early manifestations of diabetes complications. If these events could be identified, those individuals most likely to benefit from immunomodulation or who are at highest risk to develop complications could be intensively treated. Some effort in this direction has already begun. For example investigators are now collaborating on proteomic projects to identify beta cell proteins that might give rise to the immune attack. Appropriate surrogate markers could dramatically enhance researchers' ability to conduct clinical trials, as well as shorten the duration of the trials.

Research Objective—Improve the Power of Diabetes Research by Utilizing Computational Biology and Bioinformatics:

- **Enhance type 1 diabetes research efforts by incorporating bioinformatics at the inception of the research effort.**

Bioinformatics is a newly emerging field that combines data storage, organization and analysis. Bioinformatics has the power to correlate genetic, biochemical, cell function, demographic, and clinical data from disparate data sets from all over the world to create a comprehensive picture of disease. It has critical applications for analyzing complex datasets generated by clinical trials of immunomodulation and their associated mechanistic ancillary studies, or for analyzing genetic samples from thousands of type 1 diabetes patients and their families. In both instances, huge sets containing data as disparate as a patient's genotype and the immune cell complement in his or her blood will be analyzed together with descriptions of the clinical and biochemical manifestations of the disease. The efforts of bioinformatics experts will be critical to isolating in these populations of research patients the significant variables that cause (or protect against) type 1 diabetes and its complications. It is expected that new hypotheses regarding the pathology of disease will be generated by searching for novel correlations within such data sets. Because of this, future data collections could benefit greatly from involving bioinformatics experts early in the study design. The ultimate goal would be to work toward interoperability, so that data stored in all these databases can be freely accessed and combined by the research community--efficiently and productively.

- **Apply computational biology to the complex systems in type 1 diabetes.**

The ability to organize complex data sets is only the first step for bioinformatics. As biology becomes increasingly sophisticated, computer models can save time and maximize the use of resources for analysis of complex systems. For example, insulin secretion involves networks of genes and metabolites interacting among all the cell types in the pancreatic islet, which in turn, receives signals from the rest of the body in the form of hormones, nutrient levels, cells, and molecules of the immune system, and nerve impulses. Computer models could help biologists predict how all of these signals are integrated to control blood glucose levels. Similarly, the development of an artificial pancreas (see Goal IV) will depend on developing algorithms that can use data on physical activity, diet, insulin administration, and glucose levels to calculate and effect fine-tuned insulin delivery.

Research Objective—Apply New Technology to the Development of Improved Animal Models for Study of Type 1 Diabetes:

- **Develop models needed to identify cellular and molecular pathways influencing beta cell formation and function.**

Cell-based therapies designed to replace the beta cells lost due to immune-mediated destruction require a firm understanding of their developmental paths and fates, as well as a ready source of expanded cells for treatment. In pursuit of this goal, the BCBC is producing mice bearing fluorescent tags (the "Rainbow Mouse") that illuminate the developmental path followed by beta cells as they arise and begin to populate the pancreas. Investigators are extending this approach to produce animal models capable of identifying the cellular source of newly arising beta cells, as well as the molecular pathways responsible for regulating beta cell growth and function in the adult pancreas. Through studies in these and other new

animal model systems, it may be possible to identify novel molecular targets for therapeutics development in type 1 diabetes.

- **Develop animal systems with greater fidelity to human disease to enhance pre-clinical testing and biomarker development.**

In the Type 1 Diabetes-Rapid Access to Intervention Development (T1D-RAID) program, promising new drugs are being produced and tested in existing animal models for their ability to reduce type 1 diabetes autoimmunity or diabetic complications. These pre-clinical studies are being facilitated by the activities of the Animal Models of Diabetic Complications Consortium (AMDCC), which is a research consortium that is developing new mouse models of diabetic complications. Particularly promising are new AMDCC models of diabetic cardiovascular disease, nephropathy, and neuropathy that will be of tremendous value for testing new drugs for these conditions. How closely these new models reproduce the human condition, and their ultimate utility as models for pre-clinical testing of new drugs, will be determined through the joint phenotyping efforts of the AMDCC and the Mouse Metabolic Phenotyping Centers (MMPC) consortium.

New mouse strains bearing HLA or other human disease susceptibility genes that have been identified by large genetic mapping studies such as the T1DGC, FIND, and GOKIND are providing systems to study the genetic components of human type 1 diabetes in mice. The value of these genetic studies will be dramatically enhanced by the coming availability of new immunocompromised mouse models that allow for efficient reconstitution of the human immune system in mice. This important advance will, for the first time, allow in-depth mechanistic studies in mice of human autoimmunity, transplantation, and tolerance in the context of human genetic susceptibility loci and the human immune system.

While rodent models have been and continue to be a valuable tool for dissecting mechanisms of disease and for testing new drugs, studies in fish, pigs, and non-human primates are also providing valuable insights, including for the study of islet development and transplantation. As these models are validated and come into widespread use, they will allow for improved and more predictive tests of new therapies. Moreover, models in pigs and non-human primates may be particularly valuable tools for identifying new biomarkers of disease progression that are needed to improve type 1 diabetes clinical trial design and medical care.

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Table 6. Key Research Objectives for Attracting New Talent and Applying New Technologies to Type 1 Diabetes Research

- Recruit Expertise from Diverse Fields
- Establish Non-invasive Imaging Technologies for Type 1 Diabetes Research and Diagnostic Applications
- Promote Application of Advances in Bioengineering to Type 1 Diabetes
- Foster Application of Gene Delivery and Gene Silencing Technology To Develop New Therapies for Type 1 Diabetes and its Complications
- Exploit the Potential Application of New and Emerging Technologies in Functional Genomics, Proteomics, and Metabolomics to Type 1 Diabetes Research
- Improve the Power of Diabetes Research by Utilizing Computational Biology and Bioinformatics
- Apply New Technology to the Development of Improved Animal Models for Study of Type 1 Diabetes

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APPENDIX B: ACRONYMS AND ABBREVIATIONS

Organizational Components

ADA	American Diabetes Association
CDC	Centers for Disease Control and Prevention
CMS	Centers for Medicare and Medicaid Services
HHS	Department of Health and Human Services
DMICC	Diabetes Mellitus Interagency Coordinating Committee
FDA	Food and Drug Administration
JDRF	Juvenile Diabetes Research Foundation International
NCRR	National Center for Research Resources
NEI	National Eye Institute
NHGRI	National Human Genome Research Institute
NHLBI	National Heart, Lung, and Blood Institute
NIAID	National Institute of Allergy and Infectious Diseases
NICHD	National Institute of Child Health and Human Development
NIDCR	National Institute of Dental and Craniofacial Research
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NINR	National Institute of Nursing Research
NLM	National Library of Medicine

Research Programs

AMDCC	Animal Models of Diabetic Complications Consortium
BCBC	Beta Cell Biology Consortium
CCITC	Cooperative Clinical Islet Transplantation Consortium
CITR	Collaborative Islet Transplant Registry
DASP	Diabetes Autoantibody Standardization Program
DCCT	Diabetes Control and Complications Trial
DirecNet	Diabetes Research in Children Network
DPT-1	Diabetes Prevention Trial-Type 1
DRCR.net	Diabetic Retinopathy Clinical Research Network
EDIC	Epidemiology of Diabetes Interventions and Complications
FIND	Family Investigation of Nephropathy and Diabetes
GoKinD	Genetics of Kidneys in Diabetes Study
ITN	Immune Tolerance Network
NHPCSG	Non-Human Primate Transplantation Tolerance Cooperative Study Group
SEARCH	SEARCH for Diabetes in Youth Study
T1DGC	Type 1 Diabetes Genetics Consortium
TEDDY	The Environmental Determinants of Diabetes in the Young
TrialNet	Type 1 Diabetes TrialNet
TRIGR	Trial to Reduce IDDM in the Genetically at Risk

Other Acronyms and Abbreviations

ARB	angiotensin receptor blocker
ACE	angiotensin converting enzyme
AGE	advanced glycation endproducts
ARE	antioxidant response element
BMI	Body Mass Index
CGMs	continuous glucose monitors
ChIP	chromatin immunoprecipitation
DMK	dystrophia myotonica kinase
ES	Embryonic Stem Cell
FACS	fluorescence activated cell sorting
fMRI	functional magnetic resonance imaging
GAD	glutamic acid decarboxylase
HAAF	Hypoglycemic-Associated Autonomic Failure
HbA1c	hemoglobin A1c
HLA	Human Leukocyte Antigen
HMGB1	high mobility group box 1
HTS	high-throughput screening
IDDM	insulin-dependent diabetes mellitus
IGRP	islet specific glucose-6-phosphatase catalytic subunit related protein
INS	insulin gene
LDL	low-density lipoprotein
Mbp	mega base pairs
MHC	major histocompatibility complex
mRNA	messenger RNA
miRNA	micro RNA
siRNA	small interfering RNA
MLI	NIH Roadmap Molecular Libraries Initiative
MODY	Maturity Onset Diabetes of the Young
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NIP	Nutritional Intervention to Prevent
NKT	natural killer T cells
NMR	nuclear magnetic resonance
NOD	non-obese diabetic
PET	positron emission tomography
PTPN22	protein tyrosine phosphatase N22 gene
RAGE	receptor for advanced gylcation endproducts
RNAi	RNA interference
ROS	reactive oxygen species
SACO	serial analysis of chromatin occupancy
SNP	single nucleotide polymorphism
VEGF	vascular endothelial growth factor
VNTR	variable number of tandem repeats region